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6690

Reactions of Peptones with Metallic Sodium in Liquid Ammonia.

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From the Northwestern University Medical School.

We have previously reported[1] that certain proteins, amino acids, dipeptides, and diketopiperazine are acidic in liquid ammonia, and that they react with metallic sodium to liberate hydrogen with the formation of the corresponding ammono salt. We have also studied the action of sodium upon peptones under similar conditions. The following peptones were used: Armour's Meat Peptone, Merck's Meat Peptone, Witte's Peptone, and silk peptones prepared by treating silk fibroin with 70% sulfuric acid at room temperature for one, 2, 3, 4, and 10 days. We were interested in comparing the properties of peptones in liquid ammonia with the properties of proteins, amino acids, dipeptides, and diketopiperazines, and in comparing the properties of the various peptones with each other.

The procedure was essentially the same as described previously. Peptones are somewhat soluble or are dispersed in liquid ammonia. The solubility of silk peptones varies with the extent of digestion, passing through a maximum on the third day.

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[1] Miller, C. O., and Roberts, R. G., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 535.

In general, we have found that the peptones, Armour's, Merck's, Witte's, and 2 and 3 day silk peptones behave more like diketopiperazine than like proteins, amino acids, or peptides. When the quantity of hydrogen liberated by a definite amount of dried peptone is plotted against the amount of sodium that is brought into reaction, the curves for these peptones resemble closely the curve for diketopiperazine. With small amounts of sodium, relatively smaller quantities of hydrogen are liberated and greater proportion of the hydrogen is taken up by the peptone. With increasing amounts of sodium, there is a relative increase in the quantity of hydrogen liberated. The maximum is reached when the proportions of 4 gm. atoms of nitrogen are treated with 7.3 gm. atoms of sodium with the liberation of 1.2 gm. atoms of hydrogen. If peptones contained only free carboxyl groups, free amino groups and acidic imide groups, and if there was no reduction taking place, as is the case with a dipeptide, then for each 4 gm. atoms of nitrogen present in imide combination, 4 gm. atoms of hydrogen should be liberated. Since the number of carboxyl groups is approximately equal to the number of amino groups, then the N:Na:H should be 1:1:1. We did not find the imide group of dipeptides to be acidic although the imide group of N-methyl acetamide liberates 1 gm. atom of hydrogen. It is possible that the imides of diketopiperazines are acidic in the lactam form or through tautomerization to the lactim form.

In the case of the silk peptones which were hydrolyzed by 70% sulfuric acid at room temperature for 1, 2, 3, 4, and 10 days, there is a marked difference in the maximum quantity of hydrogen which is liberated when they are treated with an excess of sodium. Calculated for 4 gm. atoms of nitrogen, native silk gives 2.70 gm. atoms of hydrogen, 1 day peptone 2.35, 2 day peptone 1.07, 3 day peptone 1.15, 4 day peptone 2.63, and 10 day peptone 2.87. It is interesting to note that the change in solubility or dispersion with length of digestion period parallels this curve. Qualitative observations show that the rate at which hydrogen is liberated increases during the first 3 days, but remains constant thereafter. Furthermore, when glycine, tyrosine, and alanine are mixed in the proportions in which they are present in silk and treated with excess sodium in liquid ammonia, they do not liberate 4 gm. atoms of hydrogen for 4 gm. atoms of nitrogen, but only 2.86 gm. atoms of hydrogen. This is the same quantity that was liberated by the 10 day peptone. This might suggest that other parts of amino acids beside the carboxyl and amino groups play a part in ring or chain formation, such as the phenolic group of tyrosine. We have shown that the phenolic group in tyrosine reacts with sodium in liquid ammonia to the extent of 25% of the number of groups present.

It is interesting to note that there is first a fall and then a rise in the total amount of hydrogen liberated, with a final value approaching the theoretical value for complete hydrolysis. Comparison of the curves for Armour's, Merck's, and Witte's peptone suggest that the difference between the curves may be due to the extent of hydrolysis, in addition to the type of protein used. We think of two possible explanations for the differences noted in the properties of the silk peptones. It is possible that with decreasing length of the peptide chain, there is an increased ease with which the imide group may be reduced. The other possible explanation is that as hydrolysis of the silk proceeds, either rings are formed or are liberated in such a way that they become more reactive for awhile, which is followed by the hydrolysis of the rings to give peptides and amino acids progressively.

While we think that our investigations indicate the presence of rings in the proteins and peptones that we have studied, we wish to obtain more data before forming conclusions.

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Effect of Tyrosine on Botulinum Toxin.

WILLIAM BURROWS. (Introduced by N. Paul Hudson.)

From the Department of Hygiene and Bacteriology of the University of Chicago.

We noted, in a study of the growth of *Clostridium botulinum* on synthetic mediums,[1] considerable irregularity of toxin production, particularly among the type B strains.

A number of possible explanations for this phenomenon were considered. One which seemed most plausible arose from the work of Kempner and Schepilewsky,[2] who, stimulated by the work of Wassermann and Takaki[3] on the detoxification of tetanus toxin with brain emulsions, reported that brain emulsions and a water-insoluble component of brain would neutralize botulinum toxin. In a search for specific chemical compounds which would have this effect, they tested tyrosine, since Phisalix[4] had reported that the amino-acid would neutralize snake venom. They presented evidence indicating that tyrosine would neutralize botulinum toxin. This neutralizing capacity was quite limited, for white mice could be protected against only 3 MLD of toxin. This protection, however, was manifested not only when tyrosine and toxin were injected simultaneously, but also when the injection of tyrosine was some 24 hours before that of toxin.

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[1] Burrows, W., *J. Inf. Dis.*, 1933, **52**, 209.

[2] Kempner and Schepilewsky, *Z. f. Hyg.*, 1898, **27**, 213.

[3] Wassermann and Takaki, *Ber. klin. Wchschr.*, 1898, **1**.

Since most of the synthetic mediums referred to above contained tyrosine to the limit of its solubility, 3 possibilities were considered: (1) these quantities of tyrosine interfered with the formation of toxin; (2) the tyrosine present had a deleterious effect on formed toxin; and, (3) tyrosine injected into mice incidental to the injection of toxin protected the animals in some unknown way. We therefore tested these possibilities experimentally. We have used phenylalanine in addition to tyrosine because of the close structural relationship of the two.

The organisms were grown in beef heart medium saturated with tyrosine and in similar mediums containing phenylalanine in the same amount, *i. e.,* 40 mg. per 100 cc. of medium. We have also used mediums containing both amino-acids. In only 2 experiments in a series of 10 were there significant differences in toxin titrations between the tyrosine-containing mediums and control mediums to which tyrosine had not been added. These lowered titres were found only in type B cultures in the tyrosine mediums. Phenylalanine produced no effect whatever.

Other experiments were carried out in which the protection tests of Kempner and Schepilewsky were repeated. We have also incubated mixtures of toxin and tyrosine for periods ranging from 30 minutes to 2 hours. These experiments indicated quite definitely that injections of tyrosine, either simultaneous with the injection of toxin or 24 hours previously, would not protect mice against 3 MLD of toxin. Tyrosine was injected in quantities of 50 to 100 mg. It was also apparent from these experiments that incubation of saturated tyrosine solutions for these periods of time with toxin had no deleterious effect on the toxin itself.

Coleman,[5] in a study of peptone therapy, repeated the work of

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[4] Phisalix, *Compt. Rend.,* 1898, p. 413 (quoted by Kempner and Schepilewsky).

[5] Coleman, *J. Inf. Dis.,* 1924, **34,** 614.

Kempner and Schepilewsky on brain emulsions and was unable to confirm their results.

Summary. We have failed to protect mice against small amounts of botulinum toxin by the injection of tyrosine and have not been able to confirm the work of Kempner and Schepilewsky. We feel that the suggestive results of 2 of our experiments on the effect of excess quantities of tyrosine on the formation of botulinum toxin are not significant in the light of the other experiments described here. We have also found that neither tyrosine nor phenylalanine had a deleterious effect on formed toxin when mixtures of the amino-acids and the toxic material were incubated together.

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Experimental Production of Gastric and Duodenal Ulcers in Dogs in Cinchophen Poisoning.

T. P. CHURCHILL AND D. O. MANSHARDT. (Introduced by J. P. Simonds.)

From the Department of Pathology, Northwestern University Medical School.

Churchill and Van Wagoner[1] attempted to produce acute yellow atrophy of the liver by feeding cinchophen to dogs. Functional and histological damage was produced with large doses, but this did not simulate acute yellow atrophy. Numerous acute gastric and duodenal ulcers were observed at autopsy. Smaller doses of cinchophen gave definite functional damage of the liver, but histological damage was irregularly produced.

A total of 25 dogs were given cinchophen in varying doses from 22 mg. per kilo to 27 times that amount. In this series acute and chronic gastric ulcers were found in over 80% of the dogs used. These ulcers were typical grossly and microscopically. These results were published and the following possibilities of the mechanism of the production of these ulcers were listed[2]: 1. Cinchophen may have a direct toxic action on the gastric mucosa, combined with digestion by the gastric secretions. 2. The drug may combine with the mucin of the stomach and thus remove the normal protection of the gastric mucosa. 3. It may act on the autonomic nervous system and induce erosions by its neurogenic effect. 4. Cinchophen may influence the secretion of gastric juice, either directly or through the endocrine system. 5. The drug may have a general toxic action which affects the stomach directly or by causing metabolic disturbances as a result of its effect on the liver or pancreas. 6. The nutritional disturbances resulting from the anorexia that follows the ingestion of cinchophen may be a factor in the production of ulcers.

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[1] Churchill and Van Wagoner, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 581.

[2] Churchill and Van Wagoner, *Arch. Path.*, 1932, **14**, 860.

Further experimentation is in progress to limit these possibilities by attempting to prove or eliminate certain factors.

Well nourished, apparently healthy dogs were used. Three dogs were operated: A loop of jejunum was brought to the surface and anchored. The continuity of the intestine was not disturbed. These dogs were allowed to recover from the operation, and, upon recovery, cinchophen suspended in olive oil was injected hypodermatically through the intestinal wall. The daily dose of cinchophen was calculated so that each dog received 220 mg. of cinchophen per kilo. (The human dosage can be figured as 22 mg. per kilo per day.) The dogs were fed a meat, cereal mixture by mouth. When the stools became persistently bloody the dog was sacrificed.

Dog No. 1-A, weighing 16 kg., received a daily dosage of 3.52 gm. of cinchophen, a total of 22 doses, showed persistent bloody stools and was sacrificed. Autopsy showed a large chronic gastric ulcer 4x2.5 cm. bordering on the pyloric ring and extending proximally into the lesser curvature of the stomach. No ulcers were found at the point of injection.

Dog No. 2-A weighed 4.05 kg. was operated on January 14, 1933, and started on cinchophen January 24, 1933. Daily doses of 220 mg. of cinchophen were given for 10 days. The dog died on February 2, 1933. Autopsy revealed a massive hemorrhage into the stomach. A large pre-pyloric ulcer 1.5x2 cm. on the posterior wall near the lesser curvature had perforated and the opening was sealed by omentum. There was no blood or pus in the abdominal cavity. The site of injection was 2½ feet from the pyloric ring.

Dog No. 3-A weighed 5.15 kg. at operation. After recovery the animal weighed 7.8 kg. Twenty doses of 15.7 gm. each were given daily. At this time persistent tarry stools appeared and the dog was sacrificed. Autopsy revealed an emaciated, very weak dog, weighing 4.93 kg. An ulcer was found at the superior portion of the pyloric ring. There was no further ulceration in the gastro-intestinal tract. At the site of injection there was a large intramuscular abscess. The regional lymph nodes were enlarged. The spleen showed numerous elevated, hard, pale, grey-white nodules, 0.5 cm. or less in diameter. The gum of the lower right side of the mouth showed a large ulceration with exposure of the bone. The site of injection was 4 feet from the pyloric ring.

Microscopic sections showed typical chronic gastric ulcers. The large intramuscular abscess contained a large amount of fat and many large, clear cells with vesicular nuclei. The lymph nodes and nodules in the spleen contained a definite hyperplasia of the reticulo-endothelial cells.

Summary. These experiments have allowed us to inject the drug into the gastro-intestinal tract without passing through the stomach, and should rule out any local toxic effect of cinchophen in the stomach. No ulcers developed in the mucosa of the intestine.

Conclusion. Gastric ulcers that are produced in dogs by the injection of cinchophen are not due to a local toxic action on the gastric mucosa.

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Source of Fat Found in the Thoracic Duct Lymph in Fasting.

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From the Department of Physiology and Pharmacology, Northwestern University Medical School.

Thoracic duct lymph of fasting dogs contains 0.3 to 1.3% total fatty acids.[1] Further observations showed that with the continuous collection of the lymph under anesthesia, there is usually a spontaneous decline in the lipid content, amounting to 50% or even more in 4 hours. Injection of pilocarpine or secretin is followed by an increase of the lipid content of the lymph up to or considerably above the initial level, the maximum effect appearing about 4 hours after the injection. In enterectomized animals, the initial lipid content is low and does not rise following pilocarpine; nor does pilocarpine cause a rise of lipids in the lymph of dogs with common bile duct fistula.

Method. 26 dogs were fasted 3 to 8 days. Cannulation of the thoracic duct was done under nembutal anesthesia. For the preparation of the enterectomized animals, the bowel was removed from the recto-sigmoid junction to the midportion of the duodenum, the free end of the duodenum being brought to the outside through a stab wound. The animal was allowed to recover and was fasted several days, fluid being given twice daily subcutaneously. The dose of pilocarpine was 1 mg. per kilo and that of the secretin 60 dog units total intravenously. Total fatty acids and cholesterol were determined by Bloor's oxidation method.

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[1] Rony, H. R., Mortimer, B., and Ivy, A. C., *J. Biol. Chem.*, 1932, **46**, 737.

The finding that the thoracic duct lymph of fasting enterectomized animals is clear, and low in lipids indicates that the principal source of the milky lymph in fasting is the bowel. Furthermore, the effect of pilocarpine and secretin suggests that the lipids of the lymph come from the lumen of the bowel; in the first place when the bile is shunted to the outside, the lipid content in the lymph is low and does not rise with pilocarpine; in the second place, the delay in the appearance of the maximum lipid content after pilocarpine and secretin is consistent with the known effects of these agents on intestinal secretions. Whether pilocarpine and secretin increase the lymph fat by their effect on bile and pancreatic juice excretion or primarily by actual increase of lipid secretion into the lumen of the bowel, or both these factors together, we cannot say definitely.

These findings are in partial accord with the work of Bloor and Sperry on lipid excretion. In comparing the amounts of lipids found by us in the lymph in fasting with those found by Bloor and Sperry in the feces of sham-fed animals, it appears that from 50 to 70% of the total lipids excreted into the lumen of the bowel is reabsorbed. This extensive reabsorption along with other considerations suggests that the lipid excretion in fasting may serve other purposes than lubrication or elimination of waste lipid material as advanced by Bloor and Sperry. Possibly the purpose of this phenomenon is to subject to hydrolysis and resynthesis some of the fat mobilized in fasting, in order to modify it to a form suitable for utilization.

The principal source of the lipids in thoracic duct lymph in fasting is the bowel. In fasting, fat is being excreted from the blood by the mucous membrane of the bowel into the lumen of the bowel, where a large part of it is reabsorbed.

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Acidification of Bacterial Suspensions Containing Formaldehyde.*

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From the Department of Medicine, the University of Chicago.

It is well known that the rate of bacterial autolysis as measured either by morphological disintegration or by chemical determination of the accumulating split-products, varies widely among the pathogens. No mention could be found, however, of this process being accelerated by an aldehyde.

Certain strains of the gram-negative diplococci (thrice washed in saline) have been found to become acid in suspensions containing appropriate concentrations of formaldehyde. While most of these observations were made on bacterial suspensions equivalent to 0.25% dry weight of organisms, the reaction was found to be roughly proportional to the concentration of bacterial material. The acidification was measured by the frequency with which small additions of N/10 NaOH were required to bring the reaction of the suspensions back to pH 7.2. It was most rapid in concentrations of 0.7-2% formaldehyde, was much slower (about one-eighth the maximum) in 4% and failed to occur in 8% formaldehyde. It was more rapid at 37°C. than at room temperature.

No acidification took place when, before adding formaldehyde, the suspension was boiled for 1 minute, heated to 75°C. for 5 minutes, or 60°C. for 20 minutes. A temperature of 50°C. for several days did not inhibit the action noticeably. The presence of 10% alcohol reduced the action and 20% alcohol prevented it altogether. Heavy metal showed an inhibitory property, *e. g.,* 0.1 mg. of silver nitrate per 10 cc. inactivated the mixture. 0.001 mg. potassium cyanide per cc. of suspension prevented the reaction.

Suspensions which had been rendered alkaline to phenolphthalein for a few hours failed to become acid. A suspension started at pH 7.2, to which no alkali was added during 7 days at 37°C., developed an acidity of pH 3.2.

Accompanying the acidification of the formolized suspensions is an increase in the non-protein nitrogen as compared with the control suspensions. This was determined on the sodium tungstate-tungstic acid filtrates.

These observations suggest that the phenomenon is an autolytic

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* This work was supported in part by a grant from the A. B. Kuppenheimer Research Fund, University of Chicago.

digestion of cellular proteins accelerated by combination of formaldehyde with the protein-split products. It occurred in the case of 5 meningococcus strains of 7 investigated, one gonococcus of 5 examined, and a catarrhalis strain. It was not observed in the case of a single strain each of Pneumococcus (rough), Type III Pneumococcus (smooth), Staphylococcus, nor *Streptococcus hemolyticus.*

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Meteorological Influences on Leukocyte Curve.

WM. F. PETERSEN AND MAX BERG.

From the Department of Pathology and Bacteriology, University of Illinois College of Medicine.

When leukocyte counts are made at short time intervals (5 or 10 minutes) in human subjects or in experimental animals the fluctuations afford an excellent index of the autonomic balance of the splanchnoperipheral system.[1] In addition to this tide, the general leukocytic level will reflect the functional status of the bone marrow and the lymphatic system.

In the course of observations on various constitutional types and their reactions to the environment, we have made daily leukocyte counts under morning basal conditions. These revealed several interesting facts. First, that the leukocytic level is rather characteristic of the constitutional type, the pyknic in general having the most deficient bone marrow and the higher leukocytic levels.

Second, that the fluctuations in the level occur simultaneously in all individuals. While the pyknic may react more vigorously, the leptosome and the asthenic show a synchronous increase or decrease in the level, although the fluctuations may be of minor degree in such persons as might be anticipated from their general somatic reactivity.

Third, that these fluctuations are meteorologically conditioned. Inasmuch as the meteorological environment in its many components offers many individual factors that influence the organism (temperature, humidity, pressure, ionization, etc.) an arbitrary identification of any one might be premature, but we note the association of change in the barometric pressure as particularly striking to the change in the leukocyte count.

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[1] Petersen, Wm. F., Müller, E. F., and Boikan, Wm., *J. Infect. Dis.*, 1927, **41**, 405; Petersen, Wm. F., and Müller, E. F., *Arch. Int. Med.*, 1927, **40**, 575; Arquin, S., PROC. SOC. EXP. BIOL. AND MED., 1927, **25**, 97; Müller, E. F., Petersen, W. F., and Hölscher, R., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 544.

It would appear that periods of stimulation and recovery of the bone marrow are characteristic events in our latitude. The inference might follow that we may have too much or too little of such stimulation for certain types of individuals. The clinical pictures that might follow therefrom are readily demonstrable and will be discussed elsewhere.

The appended graph illustrates the daily leukocyte count in 4 normal young men. No. 1 was a pyknic, No. 2 a mixed athletic type, No. 3 a slender leptosome strongly sympathicotonic, No. 4 a leptosome of rather unstable type.

The upper portion of the chart is made of a meteorograph. It illustrates the daily weather conditions during July and August, 1932. (1) The top line represents the daily mean wind velocity. (2) The amount of cloudiness (black bars). (3) The average daily temperature (dotted line). (4) The barograph (heavy line).

Below, labeled 1, 2, 3, and 4, are extended the leukocytic curves. It will be noted that the curve of No. 1 is the highest while the curve of No. 4 is the lowest in actual count.

Below these 4 leukocyte curves is a horizontal line with vertical bars. These indicate "weather instability" as designated by the Director of the Chicago Station Weather Bureau. Precipitation is indicated by black bars extending down just above the dates.

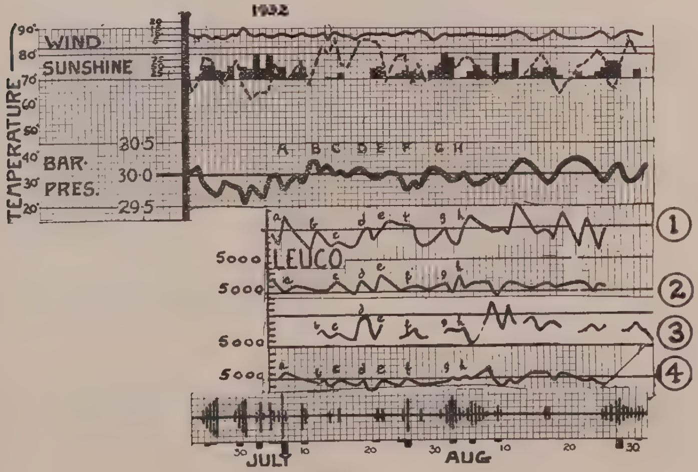

FIG. 1.

On the barograph points A to H have been designated, which correspond to periods when the leukocytic count was increasing and these letters have been carried down to the individual leukocyte curves. Of course, not every curve reflects the change to the same degree nor at quite the same time. These periods of stimulation are associated with many other evidences of general stimulation of the organisms, a mechanism which must be dealt with in subsequent papers.

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Meteorological Influences on Leukocytic Partition.

WM. F. PETERSEN AND MAX BERG.

From the Department of Pathology and Bacteriology, University of Illinois College of Medicine.

In the preceding note we have called attention to certain periodic leukocytic fluctuations which occur simultaneously in all normal individuals as well as patients.

If we analyze the differential count the leukocytic partition reveals even more clearly the periods of stimulation, both in the increase in the number of polymorphonuclear leukocytes as well as the striking periodicity in the appearance of the eosinophiles. In the normal individual this eosinophilia is limited to 2 or 3% of the total count, in the vegetatively stigmatized group of individuals, however, the count may reach 15% on such days of stimulation with a complete disappearance the following day. It is, of course, well known that the periods of stimulation apparent in the patient with pernicious anemia are heralded by a similar increase in the eosinophile count.

In the graphic presentation of the same 4 normal subjects discussed in the preceding note, a barographic tracing has been carried across the chart for each individual subject (heavy line) and the daily percentage of polymorphs is indicated in a light solid line. The number of eosinophiles is indicated by the small crosses below the barograph and basophiles are indicated by the letter B. They were only observed in subject No. 2.

It will be noted that (1) subject No. 4 has the lowest proportion of polymorphonuclear leukocytes and in this individual the periods of stimulation and recovery are of longer duration and of somewhat greater magnitude. (2) The periods of stimulation occur synchronously in all the individuals. (3) That these periods are associated with the appearance of eosinophiles so that these, too, occur simultaneously in all individuals.

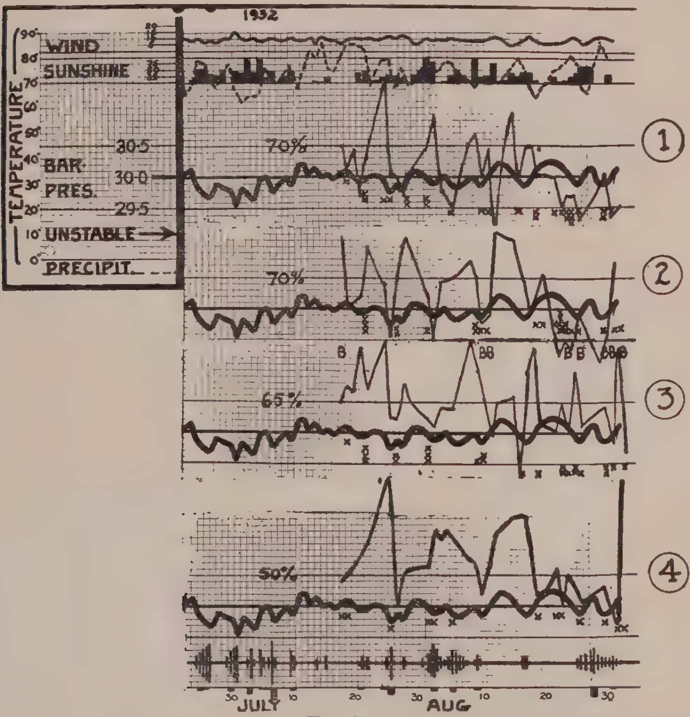

FIG. 1.

Graph illustrating relation of barometric fluctuations to the differential count. Increase in polymorphonuclear leucocytes follows the point of low barometric pressure, *i. e.,* periods of anorexemia. Points marked x indicate appearance of eosinophiles, points marked B indicate appearance of basophiles in the blood smear.

So far as we have been able to observe in the literature, only Franke[1] has noted this association of eosinophiles with weather changes.

Individual differences in the curves of the individuals here portrayed are indicative of differences in the functional condition in the hematopoietic system, with summation and fatigue more pronounced in subject No. 4. Persons of this constitutional habitus are more susceptible to the development of leukocytic pathology including the exhaustion picture of agranulocytosis or the irritation picture of leukemia. These clinical conditions will be discussed elsewhere.

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[1] Franke, Kurt, *Strahlentherapie,* 1932, **43**, 517.

6697

Determination of Serum Total Base.

WILLIAM S. HOFFMAN. (Introduced by M. M. Kunde.)

*From the Department of Physiological Chemistry, Chicago Medical School.**

Practically all the modern methods of determining serum total base are modifications of Fiske's[1] procedure for total base in urine. The modification proposed by Stadie and Ross[2] has until recently received the widest use. In this method, the serum is ashed directly without removal of phosphate, and the base in the ash, which is present as sulfate, is analyzed by the indirect titration method of determining the sulfate as benzidine sulfate. A correction is added for the amount of base present as metaphosphate.

Recently Peters and Van Slyke[3] have published a method combining the procedures of Fiske and of Stadie and Ross. The objection to the original method of Stadie and Ross is chiefly that the results obtained are too low and too variable because sulfates are not completely precipitated in the presence of phosphate and because the method of correcting for base bound to phosphate by adding the number of milliequivalents of inorganic phosphate in the serum is not accurate. Peters and Van Slyke's procedure avoids this difficulty by a preliminary removal of the phosphate as ferric phosphate by addition of ferric alum and then ammonia to precipitate the excess iron.

In the author's experience, Peters and Van Slyke's method has a number of objectionable features. First, the procedure is tedious and time-consuming. As described, a single determination requires 24 hours or longer. Secondly, the technique is so difficult and so liable to errors by loss of material that it could not be entrusted to the average hospital technician. Thirdly, the blank is high and variable. Finally, an error is produced by a precipitation of from 1 to 2 m.eq. of calcium along with the ferric phosphate and ferric hydroxide. This loss is shown in Table I, where only 75% of the calcium was recovered in the filtrates after removal of phosphate.

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* This investigation was begun when the author was a National Research Council Fellow in Medicine at Rush Medical College.

[1] Fiske, C. H., *J. Biol. Chem.*, 1922, **51**, 55.

[2] Stadie, W. C., and Ross, E. C., *J. Biol. Chem.*, 1925, **65**, 735.

[3] Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry, Vol. II —Methods, Baltimore, 1932.

TABLE I.

Comparison of Calcium Analyses on Filtrate Obtained by Author's Method with those on Filtrate by Method of Peters and Van Slyke.

Material Analyzed	Calcium per liter.		
	Direct Analysis	Author's Filtrate	Peters and Van Slyke's Filtrate
	m.eq.	m.eq.	m.eq.
Serum	5.38	5.19	3.72
"	5.30	5.22	3.43
"	5.25	5.20	3.84
Inorganic solution	6.00	6.20	5.10
" "	6.00	6.15	4.85

The author has determined serum total base by a modification of his method for urine.[4] The procedure used avoids the aforementioned objections and combines accuracy with simplicity of technique. This is all the more important in that the clinical significance of serum total base determinations has become so well recognized that it is desirable to have a method available for the routine hospital chemist.

Method. Reagents. Ferric Chloride. Four cc. of a stock solution of 10% $FeCl_3$ in 0.2 N HCl are diluted to 100 cc.

Ammonium acetate-ammonium hydroxide solution. One gm. of ammonium acetate and 73.5 cc. 0.1 N NH_4OH are made up to a liter.

Benzidine solution. In a 250 cc. volumetric flask, 4 gm. of benzidine of highest purity are added to 45 cc. of normal HCl, diluted with water to 150 cc. and agitated until dissolved. The solution is made up to the mark and filtered. Its titration equivalent is determined as follows: 2 cc. of the benzidine solution are diluted with exactly 15 cc. of water and filtered through an ashless filter paper. 15 cc. of the filtrate are titrated with 0.02 N NaOH to neutrality to phenolphthalein. The titration figure obtained is multiplied by 17/15 to give the titration equivalent of 2 cc. of benzidine solution. The solution should be refiltered every few days and restandardized. Sulfuric acid 4.0 N. Phenolphthalein, 1% in alcohol.

Sodium hydroxide 0.02 N is prepared from concentrated, carbonate-free sodium hydroxide by dilution with CO_2-free water. If used in an automatic "refill" burette, its container should be protected from CO_2 and moisture by a soda lime tube. It should be frequently restandardized.

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[4] Hoffman, W. S., *J. Biol. Chem.*, 1931, **93**, 787.

One cc. of serum, prepared carefully to avoid any hemolysis of red cells, is measured into a 25 cc. volumetric flask and diluted with about 10 cc. of water. To it are added first one cc. of ferric chloride solution and then one cc. of ammonium acetate-ammonium hydroxide solution, and then water to the mark. The contents are thoroughly shaken and poured into a large (and preferably long) Pyrex tube, which is then heated in a free flame until the first indication of boiling. At this point, there is seen a flocculent brownish yellow coagulum which contains all the protein and fat, all the phosphate, both inorganic and organic, as the ferric salt, and all the excess of iron as the basic ferric acetate. This material is immediately filtered through an ashless filter paper in a 5 cm. funnel into a 25 cc. test tube. If the proportions of reagents have been correct, the filtration is very rapid, and the filtrate is water clear. If, however, the filtration of 20 cc. of this liquid requires more than one minute, and if the filtrate is slightly cloudy, then the quantity of reagents must be adjusted. Usually this can be accomplished by using a trace more or less than the stipulated one cc. of ammonium acetate-ammonium hydroxide reagent. One or two trials will usually suffice to discover the optimum proportions; and once the required quantity is determined, it can be used indefinitely, provided that the ammonia content of the reagent is not allowed to decrease.

The filtrate is cooled under the tap to room temperature. Twenty cc., equivalent to 0.8 cc. of serum, are transferred to a 25 cc. lipped platinum crucible and treated with one cc. of 4 N H_2SO_4. The contents are evaporated on a steam bath until charring occurs, which usually requires about one and a half hours. The crucible is now transferred to a Rogers ring burner equipped with a platinum wire sling. A small flame is allowed to play over the rim of the crucible until a gentle ebullition occurs. After several minutes the liquid begins creeping up the sides of the crucible. The flame is now increased but still kept at the rim, so that evaporation takes place from the sides of the vessel without active boiling. At this point, there is little danger of loss, and the flame can be slowly increased until the upper fourth of the crucible is at a dull red. Soon there is left only a minute quantity of a viscous liquid on the bottom of the crucible which is evaporated by allowing the flame to hit lower and lower. When all the liquid is evaporated, the full force of an oxidizing flame is allowed to play over the whole crucible, keeping it at red heat for one minute. Thus the last traces of carbon are oxidized, and all sulfuric acid is removed. The whole ashing procedure can be completed in about 10 minutes.

Into the cooled crucible, exactly 15 cc. of water are measured, followed by exactly 2 cc. of the benzidine solution. The mixture is thoroughly stirred with rubber-tipped glass stirring rod until it is certain that all the inorganic sulfate has been dissolved and reprecipitated as benzidine sulfate. The latter is filtered off on an ashless filter. 15 cc. of the filtrate are titrated to neutrality to phenolphthalein with 0.02 N NaOH.

A blank determination is run on the reagents at frequent intervals. Its value is usually less than 0.05 cc. of 0.02 N NaOH.

The calculation can be best illustrated by an example. The final titration required 10.60 cc. of 0.02 N NaOH. The blank was 0.05 cc. 2 cc. of benzidine had a titration equivalent of 18.30 cc. of 0.02 N NaOH. $(18.30 - .05 - (10.60 \times 17/15) \times 5/4 \times 1/50 \times 1000) = 156$ m.eq. per liter of serum. The whole determination can be completed in little more than 2 hours.

The results obtained are about 1% too high, because of the slight change in volume during the removal of phosphate and protein at the boiling point. If, therefore, 1% is subtracted from the figure obtained by the above calculation, the correct value for serum total base concentration is obtained.

Table I shows that no calcium is lost in the precipitation of phosphate. Table II illustrates the accuracy of the determinations on inorganic solutions. In Table III are presented the results of determinations of serum total base on 15 normal men and women by both the author's method and that of Peters and Van Slyke. The average uncorrected value obtained by the author's method was 156.9 m.eq. per liter, as compared with 154.2 by the method of Peters and Van Slyke. If a correction of 1% is subtracted from the former and if 1% is added to the latter to correct for the lost

TABLE II.
Determination of Total Base in Inorganic Solutions.

Inorganic Solution m.eq. per liter			Total Base per liter	
			Author's Method	Peters and Van Slyke's Method
			m.eq.	m.eq.
Na	145		156.1	152.5
K	5	Total	156.1	154.7
Ca	6	156	156.6	154.7
P	15		156.6	155.5
			156.1	153.3
			157.5	155.2
Aver.			156.5	154.3

TABLE III.
Determination of Serum Total Base in Normal Serum.

		Total Base		
Case No.	Age and Sex	Author's Method*	Peters and Van Slyke's Method*	Stadie and Ross's Method
		m.eq. per liter	m.eq. per liter	m.eq. per liter
1	M 25	157.2	154.0	152.6
2	M 23	156.6	153.8	151.8
3	M 23	155.4	152.8	
4	M 32	157.5	151.0	
5	F 24	154.6	154.0	
6	F 28	156.1	155.0	
7	M 24	158.0	156.6	
8	M 25	158.5	155.7	
9	F 35	156.6	154.0	150.4
10	M 28	155.5	153.2	150.2
11	M 22	154.2	152.8	149.8
12	M 35	160.2	157.5	154.6
13	M 22	159.6	154.6	153.2
14	F 22	156.9	154.0	
15	M 24	156.6	153.8	
Aver.		156.9	154.2	
Min.		154.2	151.0	
Max.		160.2	157.5	
Corrected Aver.*		155.3	155.7	

* The values given are uncorrected. To obtain the correct value 1% is subtracted from the value by the author's method and 1% is added to the value by Peters and Van Slyke's method.

TABLE IV.
Recovery of Base Added to Serum.

No.	Total Base in Serum	Added Base as K_2SO_4	Total Base Calculated	Total Base Found
	m.eq. per l.	m.eq. per l.	m.eq. per l.	m.eq. per l.
Exp. A.				
1	158.8	4.6	163.4	163.9
2	158.8	13.8	172.6	171.8
3	158.8	23.0	181.8	182.3
Exp. B.				
1	156.1	4.6	160.7	161.6
2	156.1	13.8	169.9	171.0

calcium, the results agree. Table IV presents data showing the accuracy of recovery of added base to serum.

6698

Action of Certain Drugs on Isolated Intestinal Transplants.

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From the Departments of Surgery and Physiology, University of Illinois College of Medicine.

By an operative procedure previously described segments of small intestine were isolated from the remainder of the intestinal tract and transplanted onto the surface of the abdomen.[1] By applying electrodes attached to a string galvanometer to the segment, waves of altered electrical potential were obtained. Previous studies have shown that these potential changes probably arise from some cause within the segment itself because when all original connections with the central nervous system have been severed they persist in practically an unaltered form. The two most likely sources of this phenomenon are the intestinal musculature and the intrinsic nervous tissue which consists of the plexuses of Auerbach and Meissner. Gross observations and simultaneous myograms and electrograms have shown that there is a definite time relationship between muscular contractions and the electrograms.[2] However, the waves of altered electrical potential continue when no record of muscular activity can be obtained. In an effort to obtain further information of the nature and origin of the electrograms a series of drugs were administered intravenously and their effects noted. These are briefly summarized here.

Epinephrin was followed by a complete cessation of the myogram within 10 seconds but the waves of altered electrical potential persisted. Alvarez[3, 4] and his coworkers have noted similar effects of this drug. This may be explained by the stimulating action of epinephrin on the receptive mechanism of the sympathetic system, thus increasing sympathetic inhibition. The persistance of the electrogram does not rule out the possibility of myogenic origin. Physostigmin (0.1 mg. per kg.) was followed within 30 seconds by an increase in degree and duration of the contractile phase of the myogram but no marked change in the electrogram. Atropin was followed by a rapid diminution in the strength of muscular contractions until they became imperceptible, but again the electrograms persisted. As both these drugs are thought to act upon the peripheral distribution of the parasympathetics, the former stimulating and the latter paralyzing, and as neither of them greatly altered the electrograms, it suggests that the peripheral sympathetics have a stimulating influence on the strength of contractions but do not initiate or greatly influence the electrogram.

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[1] Puestow, C. B., *Arch. Surg.*, 1932, **24**, 565.

[2] Puestow, C. B., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 901.

[3] Alvarez, W. C., and Mahoney, L. J., *Am. J. Physiol.*, 1922, **59**, 421.

[4] Berkson, J., Baldes, E. J., and Alvarez, W. C., Proc. Soc. Exp. Biol. and Med., 1932, **30**, 205.

An effort was made to paralyze all the nervous tissue in the segment. If one could be certain of this he could determine whether the altered electrical potential arose in nervous or muscular tissue. A 1% solution of nicotine applied to the surfaces of the segment and injected into its wall did not totally suppress the electrogram. However, this dosage may not have been sufficient to eradicate nervous influence. Berkson,[4, 5] working with isolated strips of rabbit intestine found that when nicotine was added to the solution in which the intestine was immersed there was an alteration of both mechanical and electrical records followed later by vigorous, rhythmic muscular contractions but no manifestation of electrical activity. Cocaine in strengths up to 10% was applied to the mucosa and outer surface of the intestinal segments. The electrograms and myograms persisted. Ergotamin in large doses is said to paralyze the sympathetics and stimulate smooth muscle. Therefore if used with atropin both sympathetics and parasympathetics should be paralyzed. By using these 2 drugs together the myograms and electrograms were increased in amplitude.

Our knowledge of the exact action and dosage of these drugs is too uncertain to permit the establishment of definite deduction from their use. However, in conjunction with other observations these experiments suggest the close relationship of muscular activity to the electrograms obtained from transplanted intestinal segments and the possibility of a myogenic origin of the potential changes.

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[5] Berkson, J., *Proc. Staff Meet. Mayo Clinic*, 1933, **8**, 24.

6699

On the Question of Equipotentiality of the Spurs in the Leghorn Fowl.*

A. W. KOZELKA. (Introduced by F. R. Lillie.)

From the Whitman Laboratory of Experimental Zoology, University of Chicago.

In previous communications[1, 2] it has been shown that the spurs of the two sexes do not react equally to the sex hosts once the chick is hatched. Spurs from either sex grafted to the male host were for the most part of the male type but on the female each was characteristic of its sex. This might be explained either on the basis of a conditioning of the male spur by the male hormone prior to the time of hatching or on the basis of a genetic difference in the spurs of the two sexes.

In an effort to determine whether the spurs of the female can be conditioned by the male hormone and whether a spur will continue to develop in the presence of the female hormone after growth has begun as maintained by Goodale[3] and Domm,[4] spurs from females were grafted to male hosts and after varying periods were transplanted back to the original donors. Complete records are available for 9 experiments, 7 between infant chicks and 2 between adult birds. Of the experiments between infant chicks, one spur was taken from a chick the day of hatching, 4 from day-old chicks, one from a 2-day old chick, and one from a 4-day old chick. These were allowed to grow on male hosts during periods varying from 14 to 24 days before they were transplanted back to the original donors. Between the adults, the one spur was allowed to remain on the male host 8 weeks, during which time it increased 3.0 mm. in length; the other remained 14 weeks, during which time its length increased by 3.5 mm. In all the experiments between the infant chicks, the spurs developed into normal female spurs. Those between the adults regressed slightly after they had been transplanted back to the original donors. At the termination of the experiment, which lasted 7 and 4 months respectively after the second transplantation, they

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* The material for this investigation has been secured during the writer's tenure of a National Research Council Fellowship. The expenses of this investigation were supported by the Committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. F. R. Lillie.

[1] Kozelka, A. W., *J. Heredity*, 1929, **20**, 1.

[2] Kozelka, A. W., *J. Exp. Zool.*, 1932, **61**, 431.

[3] Goodale, H. D., Carnegie Inst. of Washington, 1916, 243.

[4] Domm, L. V., *J. Exp. Zool.*, 1927, **48**, 31.

approximated the size of a normal female spur. This subsequent reduction in size of the spur was undoubtedly due to the natural wear on the spur.

It is evident from these experiments that the male hormone does not influence the female spur once the chick is hatched. Nor does it appear probable from the 2 experiments with the adult birds that the female spur continues to develop in the presence of a normal ovary once growth has begun. The fact that the spur continues to develop in the poulard even after the plumage has reverted to the female type and not in the normal female would indicate that the threshold level of the female hormone necessary to inhibit the development of the female spur is high. It may still be possible that the spur is subjected to conditioning only during an early critical period of development, at the time the primordial cells of the spur are determined. But the fact that the female spur is capable of developing at any stage in the life of the bird, would indicate that it remains in an embryonic condition and should therefore be subjected to the influence of the male hormone even after hatching. While these facts favor the theory that the difference in the behavior of the spurs is due to a genetic difference rather than to a conditioning of the male spur by the male hormone, the final answer as to the equipotentiality of the spurs in the two sexes cannot be made until critical data on the effects of the male hormone on the primordium of the female spur has been obtained. Work is now in progress on this phase of the problem.

6700

Implantation of Pineal Glands in the Leghorn Fowl.*

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From the Whitman Laboratory of Experimental Zoology, University of Chicago.

Foà[1] reported a precocious development of the secondary sex structures in pinealectomized males of the domestic fowl. The growth of the body of the cockerels was retarded during the first

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* The material for this investigation has been secured during the writer's tenure of a National Research Fellowship. The expenses of this investigation were supported by the Committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. F. R. Lillie.

[1] Foà, C., *Arch. Ital. de Biol.*, 1914, **61**, 79.

2 or 3 months following the operation, but after this period of retarded growth they attained the weight of normal adults. No noticeable effect was produced on the operated hens. Izawa[2] observed a precocious increase in body weight as well as in the size of the comb in both sexes. Christea[3] obtained contradictory results. He observed a marked retardation in the development of the secondary sex characters in male birds in which the pineal gland had been removed. The development of the body was not particularly delayed. Badertscher[4] did not observe any significant difference in the development of the secondary sex characters nor in body weight between pinealectomized birds and the controls.

In an effort to collect additional data on the function of epiphysis, implantation of the pineal gland was resorted to as a possible point of attack, a method that has proved successful in the study of some of the other endocrine glands. Because of the prevalent idea among a number of investigators that the pineal gland is concerned with the rate of maturity, pineal bodies from adults were implanted subcutaneously into infant chicks; and pineal glands from infant chicks and from embryos in the latter stages of incubation were implanted into half grown birds, birds in which the secondary sex characters showed marked sex dimorphism. In the first series of experiments 2 infant male chicks were used. The one received 14 implants from adult birds over a period of 28 days; the other, 25 over a period of 44 days. In the latter series of experiments, 2 females and 2 males were employed. The one female received 18 implants from the 43 to the 90 day after hatching; the other, 30 from the 40 to the 97 day. Of the males, one received 27 implants from the 40 to the 99 day after hatching; the other, 36 from the 55 to the 114 day. The transplantations in both series of experiments were made for the most part between related individuals, many of which were full brother and sister. Nevertheless, all the grafts were eventually absorbed, as far as could be determined, due undoubtedly in part to the development of a condition in the host that made it sensitive to the foreign protein. In general, the grafts in the infant chicks were evident for more than a week, whereas those in the older birds persisted only a few days.

The increase in body weight and in growth of the comb of the birds receiving implants differed in no significant way from those of their control brothers and sisters. It thus appears from the experiments here reported and from those of Badertscher in pinealectomy that the pineal gland is not of vital importance during puberty. From the many experiments on the extirpation of the gland in the fowl and in mammals, we may safely conclude that if the pineal gland does have a function in adult life, this function can, in the absence of the gland, be performed by other tissues in the body.

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[2] Izawa, Y., *Am. J. Med. Sci.*, 1923, **166**, 185.

[3] Christea, G., (cited by Krabbe) *Rev. Sp. Med.*, 1912.

[4] Badertscher, J. A., *Anat. Rec.*, 1924, **28**, 177.

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6701

Action of Copper and Other Elements in Iron Metabolism.

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Since work from this laboratory[1] has indicated that copper is not the only inorganic element which can supplement iron to bring about hemoglobin regeneration in the nutritional anemia of the rat, the present study was undertaken to compare the influence of copper and other elements on the iron content of the liver. The experimental procedure was very similar to that employed by Elvehjem and Sherman.[2] The tissue iron content was determined by the method described by Hanzal.[3]

The present report deals only with the influence of copper. The accompanying table shows the changes in the iron content of the various rat livers.

TABLE I.
Changes of Iron Content in Liver of Rat.

		Red Blood Cells per c.mm.				
No. Animals	Liver Fe Dry Tissue	Anemic Level	14 days	28 days	Hb Increase over Anemic Level	Remarks
	mg. per gm.	millions	millions	millions	gm. per 100 cc.	
5	0.33					Normal animals
8	0.31	2.04				Anemic ”
5	0.51	2.47	6.03		7.1	” ” +.5 mg. Fe daily 14 days
5	0.38	1.86	5.12	4.77	3.5	Same, then 14 days milk, no Fe and no Cu
5	0.17	2.12	4.42	7.19	6.0	Same, then 14 days .05 mg. Cu
5	0.11	1.94	4.71		2.4	

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[1] Myers, V. C., and Beard, H. H., *J. Biol. Chem.*, 1931, **94**, 89.

[2] Elvehjem, C. A., and Sherman, W. C., *J. Biol. Chem.*, 1932, **98**, 309.

[3] Hanzal, R. F., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 846.

As can be seen when iron is administered to the anemic animal for 14 days there is an increase in the iron content of the liver as well as an increase in the blood hemoglobin and erythrocytes. With discontinuing the iron at the end of 14 days and giving milk only the liver iron decreases as does the blood hemoglobin and erythrocytes. When copper is given at the end of 14 days, and no iron, the liver iron markedly decreases, while the blood hemoglobin and erythrocytes increase. Copper given to anemic animals reduces the liver iron to a minimum level with a coincident increase in blood hemoglobin and erythrocytes.

It may be concluded that copper when given to anemic rats can mobilize the iron stored in the liver to produce hemoglobin and increase the red blood cells.

6702

Determination of Iron in Biological Material.

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A method is proposed for the determination of iron in biological material which makes use of the reaction between iron and thioglycollic acid in an alkaline solution with the production of a purple color.

Method for blood. Measure 0.2 cc. of blood into a pipette accurately calibrated to contain 0.2 cc. and deliver it into a Pyrex test tube, 20x180 mm., calibrated at 10, 15 and 20 cc. Add 1 cc. of 10 N sulfuric acid and introduce a small piece of silica or a glass bead to prevent bumping. Heat, preferably over a hot plate to evaporate the water. As soon as the material chars, allow to cool for 45 seconds and add 4 drops of 30% hydrogen peroxide drop by drop. Continue the heating for 5 minutes after the solution becomes colorless. Allow it to cool and add 10 cc. water. Into a similarly calibrated Pyrex test tube accurately measure 1 cc. of standard iron solution containing 0.1 mg. iron and dilute with distilled water to about the 15 cc. mark. Add one drop (0.05 cc.) of thioglycollic acid to both standard and unknown, mix by lateral shaking. Run in concentrated ammonium hydroxide from a burette until the permanent purple color makes its appearance, pH of 8 to 10. Mix contents of both tubes, make up to volume of 20 cc. with distilled water and mix again by inversion. Comparison is then made in the colorimeter. The simplified calculation is 1000/R = mg. iron per 100 cc. blood. This is converted to volumes per cent of oxygen by multiplying the mg. iron by 0.4. To convert this value to grams hemoglobin, divide the volume per cent of oxygen by 1.34.

Method for urine, feces, milk, etc. A portion of material to be analyzed is evaporated to dryness, ashed in an electric muffle furnace, and the ash dissolved in 6 N hydrochloric acid. The insoluble residue is filtered off, washed, and filtrate made up to volume of 50 or 100 cc. To separate the iron from all possible interfering substances use is made of cupferron, the ammonium salt of nitrosophenylhydroxylamine, which precipitates ferric iron quantitatively in an acid solution. Measure an aliquot, 25 cc., of the acid solution of ashed material into the Pyrex test tubes used in the case of blood, add a dilute solution of $KMnO_4$ drop by drop until a permanent pink color appears (to oxidize all the iron to the ferric state). Add 5 drops freshly prepared 9% solution of cupferron. Mix well and centrifuge at a relatively high speed for 4 minutes. Decant the supernatant liquid. In case the iron concentration is very low, add another aliquot portion of the acid solution of ashed material, oxidize with $KMnO_4$ as before, add more cupferron, mix and centrifuge. The final precipitate is digested by adding 1 cc. of 10 N sulfuric acid, and heating over a microburner. When the material is well charred, add 30% hydrogen peroxide drop by drop and continue heating until a clear solution is obtained. Allow tube and contents to cool, add 10 cc. of water, one drop of thioglycollic acid, make alkaline with concentrated ammonium hydroxide, make up to volume of 15, 20, or 25 cc. The standard may consist of 0.05 or 0.10 mg. iron, depending upon the quantity of iron in the unknown. The color is developed here as in the case of blood and comparison made in the colorimeter.

The recovery of added iron to biological material is quantitative (Table I).

The use of cupferron enables one to make a quantitative separation of iron from substances which might interfere if present in high concentration. However, ortho- and pyro-phosphates do not interfere if present even in such quantities as 750 and 500 mg. respectively per each 0.1 mg. of iron.

The proportionality of color development over a wide range, 0.04 mg. to 0.50 mg. is practically perfect. The fading of the purple

TABLE I.
Recovery of Added Iron to Biological Material.

Material Analyzed	Iron per Specimen	Iron Added	Total Iron Recovered
	mg.	mg.	mg.
300 cc urine	0.074	0.040	0.119
300 ” ”	0.052	0.040	0.091
1 gm liver	5.630	1.000	6.611
2 ” ”	11.260	0.700	11.964
11 ” feces	1.670	0.500	2.180
25 ” ”	6.327	3.000	9.235
3 ” milk powder	0.056	0.050	0.108
4 ” ” ”	0.074	0.050	0.119

color reported[1, 2] does not occur when one drop of the thioglycollic acid is used in the presence of these low concentrations of iron. The color is permanent for 30 minutes. In high concentrations of iron, 1 mg. or more, there is fading with the subsequent return of purple color on shaking with air.

Thioglycollic acid is specific for both ferrous and ferric iron, as is shown by standard solutions of ferrous iron, which yield the usual red color with ammonium thiocyanate only after oxidation with $KMnO_4$ but yield quantitative results with thioglycollic acid both before and after oxidation.

6703

Effect of Testicular Hormone on Hypophysectomized Rats.

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From the Department of Biochemistry, Cleveland Clinic.

The purpose of these experiments was to analyze further the factors necessary for the maintenance of the prostate. It is well known that prostatic atrophy follows either hypophysectomy or castration. The atrophy which follows castration can be prevented by the use of testicular hormone. Moore and Price[1] believe that the prostatic atrophy which follows hypophysectomy is entirely secondary to the changes which occur in the testes. Moore has quoted unpublished experiments by Vatna that testicular extracts maintain the prostate in hypophysectomized rats. Moore believes that secretions produced by the hypophysis stimulate the functions of the gonads, that is, both germ cell production, and hormone secretion, and that the prehypophyseal hormone has no direct action on the prostate. It is not impossible, however, that under normal physiologic conditions, the prostate is supported directly by both the hypophyseal and testicular hormones. Some experimental evidence supports this latter theory.[2]

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[1] Michaelis, L., and Guzman Barron, E. S., *J. Biol. Chem.*, 1929, **83**, 191.

[2] Cannan, R. K., and Richardson, G. M., *Biochem. J.*, 1929, **23**, 1242.

[1] Moore, C. R., and Price, Dorothy, *Am. J. Anat.*, 1932, **50**, 13.

We have injected hypophysectomized rats with a hormone prepared from urine which is believed to be derived from the testes.[3] The dose was calculated to be that which would scarcely replace the normal secretion from the testes. If the hypophyseal hormone exerts a direct influence on the prostate it would be expected that, under these conditions, atrophy of the prostate would ensue. The table shows some typical findings in 3 rats that were littermates. These findings are in complete accord with Moore's theory of prostatic maintenance. The histologic appearance of the organs of these animals was even more striking than the gross appearance or weight. There seems to be no question that the prostate can be maintained by the thermostable hormone in urine in the complete absence of the hypophysis or hypophyseal secretions.

In addition to the above findings in the prostate a singularly interesting observation has been made in regard to the testes. Moore[4]

TABLE I.

Weight in Grams of Organs of Littermate Rats, Showing Effect of Injection of Male Sex Hormone into Hypophysectomized Rats.

Rat No.	49	49-B	49-A
Wt. at end of experiment	184	130	122
Testes	2.246	.393	1.603
Epididymis	.738	.159	.503
Seminal Vesicles	.573	.080	.507
Ventral Prostate	.251	.021	.206
Dorsal "	.139	.018	.071
Thyroid	.048	.026	.020
Adrenal	.028	.007	.005
Days	—	29	29
Remarks	Control	Hypophysectomized	Hypophysectomized. Injected

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[2] Graber, H. T., and Cowles, R. A., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 384.

[3] McCullagh, D. Roy, Cuyler, W. Kenneth, and Frawley, John T., *Trans. R. S. Canada, Sec.*, 1932, **5**, 183.

[4] Moore, C. R., Sex and Internal Secretions, Williams and Wilkins, Baltimore, 1932, 356.

has made the following statement: "The conception of the interactions between the gonads and hypophysis is supported by a wealth of determined facts and both the conception and the facts argue against the possibility of stimulating either gonad by administration of gonad hormones. A great many biologic and clinical procedures have been carried out on the supposition that such a stimulation would follow, but no endocrine gland has yet been proven to be stimulated by the administration of substances which it itself produces."

In our experiments, the testicular hormone maintained the testes in an apparently normal condition after the hypophysis was removed. It was impossible, either grossly or microscopically, to differentiate between the testes of the hypophysectomized animals that received injections of the hormone and those of the normal littermate controls.

6704

Intravenous Administration of Wheat Germ to Patients with Pellagra.

T. D. SPIES. (Introduced by J. T. Wearn.)

From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, and the Medical Service, Lakeside Hospital, Cleveland.

During the past 200 years various diets, drugs, and minerals have been recommended as therapeutic cures for pellagra. Yeast and wheat germ are two of the materials sometimes used in the treatment of this disease. Both of them contain fat, protein, and carbohydrate and are especially rich in the vitamin B complex. Goldberger and associates[1, 2, 3] have found that both yeast and wheat germ are efficacious in the prevention of pellagra and possess curative properties when administered orally to the pellagrins. While studying various methods of treatment we observed[4] that the mortality rate was very high in those individuals who because of oral

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[1] Goldberger, J., and Tanner, W., *U. S. Public Health Report*, 1925, **40**, 54.

[2] Goldberger, J., Wheeler, G., Lillie, R., and Rogers, L., *U. S. Public Health Report*, 1926, **41**, 297.

[3] Goldberger, J., and Wheeler, G., *U. S. Public Health Report*, 1927, Reprint No. 1181, 2383.

[4] Spies, T., *Am. J. Med. Sci.*, 1932, **184**, 837.

lesions or vomiting did not tolerate a good diet. In an unpublished study, we found that 53% of all cases with pellagra died despite the fact that they received the best of hospital treatment including a high vitamin, high caloric diet if they were able to eat it. Since many of our patients with pellagra are unable either to ingest highly nutritious food or retain it after ingestion, it seemed worth while to attempt parenteral therapy. A solution rich in vitamin B and poor in lipoids and proteins was made from wheat germ.* It was injected intravenously into several laboratory animals as a 10% solution in normal saline and when it seemed to do them no harm it was administered to the writer and 20 patients on the general medical wards without any serious ill effects. It then seemed safe to inject it likewise in patients with pellagra. Six pellagra patients showed definite improvement while receiving a restricted diet and repeated injections of the wheat germ solution. Intravenous injections of the solution were then given to 4 pellagrins with severe vomiting who at the same time were denied food. Following the injections the stomatitis improved within the next 2 or 3 days. By the end of this period the patients desired food and it did not seem justifiable to withhold it any longer. None of the individuals had further vomiting and each made an uneventful recovery.

The intravenous method is not recommended as one of choice except possibly when a patient with pellagra is unable to assimilate food by mouth. It has the contraindications of intravenous injections in general and is occasionally followed by mild chill and in one instance a transient phlebitis occurred. In reporting the use of an extract of wheat germ for intravenous administration to patients with pellagra, no claims are made concerning its efficacy as a therapeutic agent although it is true that these patients improved following its use. Many variables, such as rest in bed, the injection of large quantities of normal saline, the withholding of food from individuals who are vomiting, and the tendency toward spontaneous remissions of pellagra must all be considered before any final evaluation can be made.

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* The crude product was furnished through the courtesy of Dr. E. A. Sharp, Parke Davis & Co.

6705

Efficacy of Intraperitoneal Injection of Iron in the Nutritional Anemia of Rats.

MARGARET W. EVELETH, FRANKLIN C. BING AND VICTOR C. MYERS.

From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland.

Hart and his collaborators[1] reported that anemic rats did not recover when pure iron salts were administered unless small amounts of copper were also given. These observations have been confirmed in several laboratories, but Myers and his collaborators[2] have always been able to secure some response with iron alone.

The present series of experiments continues work previously reported designed to gain some insight into the reasons for the conflicting results. It is well-known that many factors may affect intestinal absorption of iron. We have therefore studied the effect of parenterally administered solutions of ferric chloride. Rats were made anemic by restriction to a diet of certified milk, using both the technic described by Waddell *et al*[3] and the method of Elvejhem and Kemmerer.[4] No difference in the response of the anemic animals to treatment was observed with either procedure. The animals, which were severely anemic, were injected intraperitoneally with ferric chloride solutions prepared from electrolytic iron. The stock solution of iron was diluted with warm, sterile saline solution (prepared from copper-free sodium chloride) and injected immediately after dilution, using a tuberculin syringe and chromium plated needles. The dilutions were so arranged that the amount of fluid injected into each animal was always 0.50 cc. regardless of the dosage. The acidity of the original stock solution was also adjusted so that the reaction of the material injected was between pH 2.0 and pH 2.5. To avoid excessive irritation the animals received injections only on alternate days. At regular weekly intervals hemoglobin determinations and red cell counts were made upon blood obtained by clipping the tail.

The rats consumed 20 to 30 cc. of milk daily at the beginning of the experiment, increased the intake with the instigation of iron therapy, and drank as much as 90 to 100 cc. per day after attaining a weight of about 125 gm. The rats approximately tripled their original weights, ultimately weighing over 200 gm., indicating that the milk used was of satisfactory vitamin content. The rate of growth was independent of the blood response, and practically identical in the different groups irrespective of the iron dosage. All the rats, in addition to the increase in total hemoglobin that must accompany growth, were able to raise the hemoglobin concentration of the blood to an extent depending upon the dosage of iron. With 0.5 mg., or more, of iron the hemoglobin reached the normal level of 12 to 15 gm. per 100 cc. of blood in 7 weeks or less; with 0.2 mg., the rate of regeneration was slower, but was complete in about 14 weeks. Lower dosages did not result in complete restoration within 22 weeks, at which time the experiment was discontinued. These animals attained hemoglobin concentrations of 8 to 10 gm. per 100 cc. of blood. The erythrocytes of the rats in this group, however, increased from an average initial level of 2.3 to 3.3 million per cubic millimeter of blood to 11 million and over. This peculiar condition of *polycythemia accompanied by low hemoglobin values* was manifest after 11 weeks on the curative regimen and was seen in all the animals in this group until the end of the experiment 3 months later. Although no data were obtained on the blood volume we believe this chronic condition was due to the production of red cells outstripping the rate of hemoglobin formation, which, in turn, was limited by the amount of iron administered to the animals.

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[1] Hart, E. B., Steenbock, H., Waddell, J., and Elvejhem, C. A., *J. Biol. Chem.*, 1928, **77**, 797.

[2] Beard, H. H., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 71.

[3] Waddell, J., Steenbock, H., Elvejhem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1928, **77**, 769.

[4] Elvejhem, C. A., and Kemmerer, A. R., *J. Biol. Chem.*, 1931, **93**, 189.

Further experiments were performed to ascertain the effect of added copper on the rate of regeneration observed with injected iron. Anemic rats received injections of iron, as before, the dosages ranging from 0.025 to 0.50 mg. of iron every other day. Some animals received in addition 0.025 mg. of copper in the form of copper sulfate solution, which was either injected with the iron or fed by mouth on alternate days. The total number of rats receiving iron supplements was 33; of those receiving supplements of iron and copper, the number was 18. Within an observation period of 6 weeks the rate of growth was identical in both groups, irrespective of the presence or absence of copper, or the dosage of iron. The red cells and hemoglobin of the blood also increased, the extent of hemoglobin regeneration depending only on the dosage of iron. All previous workers who have found the oral administration of pure iron salts to be effective in curing anemic rats have noted that the addition of copper causes a marked acceleration of the cure. The failure of copper to speed up the rate of hemoglobin production, when the iron is administered parenterally, indicates to us that the rôle of copper in hemoglobin production must now be evaluated in terms of iron absorption.

The milk used contained an average of 0.34 mg. of copper per liter, as estimated by the Biazzo procedure described by Ansbacher, Remington and Culp.[5] Keil and Nelson[6, 7] have reported the failure of pure iron to cure nutritional anemia, when the milk contained 0.24 mg. of copper per liter. However, in a few rats receiving the same source of milk they found that intraperitoneally injected iron was curative. Our results confirm and extend theirs, although the copper content was somewhat higher in the milk used by us. Preliminary experiments performed in this laboratory indicate that the administration of injected iron solutions also cures rats made anemic on milk containing only 0.14 mg. of copper per liter.

6706

Variations in Serum Calcium and Phosphorus During Pregnancy.

J. W. MULL AND A. H. BILL. (Introduced by V. C. Myers.)

From the Laboratories of the Maternity Hospital and the Department of Obstetrics, School of Medicine, Western Reserve University.

The serum calcium and phosphorus were followed in a series of 579 dispensary patients throughout the course of their pregnancy. 3501 determinations were made in the interval between 28 weeks before delivery and 7 weeks postpartum.

Two factors were found to influence the calcium, the development of the pregnancy and the season. As the pregnancy progressed there was a gradual decline which reached a maximum a few weeks before delivery. This decline was not so sharp, from 9.86 mg. to 9.64 mg., during the first 4 months of the year, a season of low values, as during the remaining months, 10.46 mg. to 9.90 mg., but the drop is still significant. The last few weeks preceding delivery show a slight rise in both cases, succeeded immediately after the delivery by a marked increase, to 10.20 mg. and 10.40 mg. respect-

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[5] Ansbacher, S., Remington, R. E., and Culp, F. B., *J. Ind. Eng. Chem., Anal. Ed.*, 1931, **3**, 314.

[6] Keil, H. L., and Nelson, V. E., *J. Biol. Chem.*, 1931, **93**, 49.

[7] Keil, H. L., and Nelson, V. E., *J. Biol. Chem.*, 1932, **97**, 115.

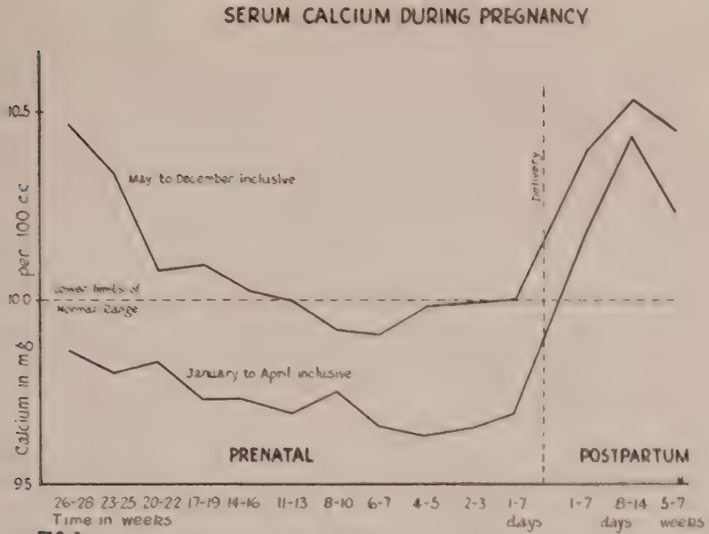

FIG. 1

ively, which was maintained to the end of the study, 5 to 7 weeks postpartum.

The seasonal effect is illustrated by the distinctly lower values found in blood drawn during the first 4 months of the year, the winter months, compared with those of the same stage of pregnancy drawn during the remaining 8 months. The 2 curves, based on averages for each season, are shown in Fig. 1. The depleted state

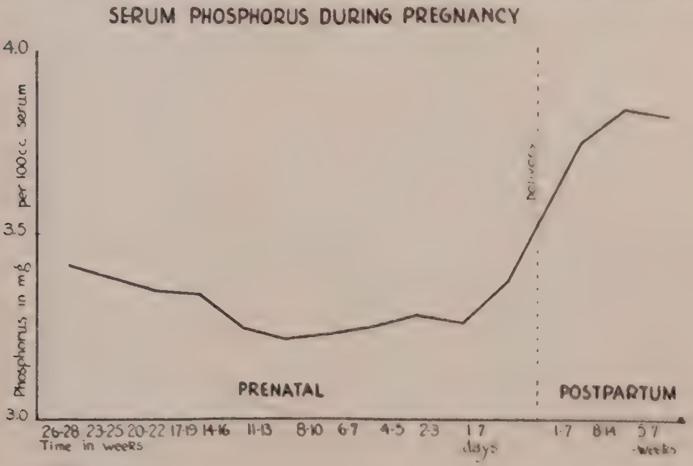

FIG. 2

found during the winter months is clearly demonstrated by the fact that the entire curve, up to the time of delivery, lies below the lower limits of the normal range, 10.0 mg. to 11.5 mg. per 100 cc. of serum, as determined by the authors.[1] The May to December averages are higher, though even these never rise above the lower third of the normal range. The differences between the curves although small at some stages, are in every case significant.

The averages of the serum phosphorus determinations are shown in Fig. 2. The gradual decline, 3.4 mg. to 3.2 mg., which reaches a maximum about 11 to 12 weeks before delivery, although clearly indicated is so slight that it can barely be considered significant. The rise to 3.85 mg. following delivery is, however, immediate and beyond question. There is no evidence of any seasonal variation.

Repeated dental examinations were made on 358 women during 3 months or more of pregnancy. Only 54 showed evidence of active decay within this time. The calcium and phosphorus were studied in 49 of these cases. Each determination on these 49 individuals, some 354 in all, was checked against the range as established by our investigation for the particular interval of the pregnancy in which it fell. Only a few were outside the normal distribution range, and of these an equal number were above and below. In no case were as many as half the determinations on any one person outside the range. There is, therefore, no connection between the teeth and the serum calcium or phosphorus.

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[1] Mull, J. W., and Bill, A. H., *J. Lab. and Clin. Med.*, in press.

Iowa Section.

State University of Iowa, February 23, 1933.

6707

Heart Weights of Normal and Anemic Animals.

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From the Iowa Child Welfare Research Station, State University of Iowa.

Cardiac hypertrophy resulting from nutritional anemia reported by Forman and Daniels[1] was based on moist heart weights. There was nothing to indicate whether the enlargement was a true hypertrophy—an increase in muscle fiber, or was caused by an imbibition of water. In the present study both moist and dry heart weights of anemic rats at different hemoglobin levels, were compared on the basis of sex, age, body weight, and body length with those of normal animals of the same sex, age, length, and weight. In an attempt to determine the fate of these hypertrophied hearts, other anemic animals were fed anemia correcting diets. When the

TABLE I.
Relation of Heart Weight to Body Weight and Body Length in Normal and Anemic Animals.

Sex	Condition of Heart	No. Animals	Age	Av. Wt.	Length	Hemoglobin per 100 cc.	Heart Wt. Moist	Heart Wt. Dry	Dry Heart Wt. Length Ratio
			days	gm.	cm.	gm.	gm.	gm.	
M	Normal	18	65.9	186	17.4	15.7	.747±.027	.170±.008	.010
M	Hypertrophied	20	65.3	138	15.5	5.6	1.061±.038	.214±.008	.013
M	Recovered	14	89.7	215	18.2	15.1	1.111±.028	.244±.007	.013
F	Normal	15	83.0	167	16.3	16	.656±.016	.151±.004	.009
F	Hypertrophied	12	65.3	107	14.5	5.8	.990±.083	.193±.017	.013
F	Recovered	8	110.2	187	17.8	16.4	.978±.028	.220±.005	.012
M	Normal	5	44	107	14.6	15.5	.504	.108	.007
F	Normal	3	45.3	95	13.7	16.3	.505	.109	.008

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[1] Forman, M. B., and Daniels, A. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 479.

haemoglobin and red cell count were again within the normal range, their hearts were compared with those of normal individuals.

The findings indicate that nutritional anemia produces a true cardiac hypertrophy, the result of increased muscle fiber. The dried heart weights were found to be distinctly heavier, whether compared on the basis of age, weight, or length, the degree of enlargement based on the dry heart weights being inversely proportional to the degree of anemia. The ratio of dried heart weight to body length was found to average approximately the same for male and female in both normal and anemic groups, although a consistently higher ratio was observed amongst the latter. During the period of observation (24 to 44 days), the animals receiving the anemia correcting diet continued to show a high degree of hypertrophy in spite of the fact that the blood haemoglobin and red cell count had returned to normal.

6708

A Simple Method of Demonstrating Blood Circulation in the Wings and Wing-pads of the Cockroach, Periplaneta americana Linn.

J. FRANKLIN YEAGER AND GEORGE O. HENDRICKSON.

From the Department of Zoology and Entomology, Iowa State College.

It has long been known that blood or hemolymph circulates in the wings of certain insects (Carus, Moseley, Brocher, and others). Most of these authors apparently observed the wing circulation with difficulty and were unable to identify completely the circulation scheme in a single insect wing at a given time. Such circulation diagrams, therefore, represent averages of a number of observations and conjectures based upon these. Brocher,[1] until recently,[2] saw the movement of individual blood cells only occasionally. Moseley[3] was unable to observe blood circulation in the forewing of the cockroach, *P. orientalis*.

The present paper reports a simple method of observing the details of blood circulation in the wings and wing-pads, as well as in the appendages and certain portions of the body, of the cockroach, *P. americana*.

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[1] Brocher, F., *Arch. Zool. exp. et gen.*, 1916, **55**, 347.

[2] Brocher, F., *Rev. Suisse Zool.*, 1929, **36**, 593.

[3] Moseley, H. N., *Quar. J. Micr. Sci.*, 1871, **11**, 389.

Circulation in the forewing may be observed by placing the animal in a depression made in a piece of balsa wood; the depression should just fit the insect and should be of such depth as to leave the dorsal surface of the body slightly higher than the surrounding wood. The head and pronotum are retained by a piece of paper (Fig. 1, *p*) pinned to the wood (*w*); the abdomen and all but the forewing (F) to be observed are held in a similar manner by a piece of dull black paper (*b*). The wing (F) should be at an angle to the black paper (*b*), upon which is placed a movable piece of white kymograph paper (*k*) with the shiny surface uppermost, so that a beam of intense light (*l*) from a low voltage incandescent lamp (*L*) can be directed downward to the edge (*e*) of the kymograph paper, as shown by the arrow in Fig. 1. The details of circulation in the portion of wing lying just above the edge (*e*) of the white paper can be observed with the low power of the ordinary compound microscope; in order to observe circulation in other regions of the wing it is only necessary to move the edge (*e*) of the white paper to a point beneath the region to be observed and then to move the entire block of wood until the new region is in the beam of light and in focus through the microscope. By thus combining transmitted and reflected light the flowing blood becomes readily visible. The visibility of the blood depends almost entirely upon the reflection of light by the circulating blood cells. Both the absence of refractive bodies (cells) in the blood and the presence of much pigment in the wing tend to prevent the detection of wing circulation.

By means of this method, the scheme of circulation has been studied in the wings and wing-pads of *P. americana*. In general, the blood flows to the body in the anal veins excepting the first (i. e., in the posterior half of the wing) and from the body in the costa, subcosta, radius, media, cubitus, and first anal veins (i. e., in the anterior half of the wing). The blood flows in all of the main veins, intermediate veins, and cross channels. The flow may be very rapid and very constant in insects in good condition, when the animal remains quiescent in a snugly fitting depression chamber. Otherwise the flow may vary greatly, may stop completely, or may reverse in direction. Copious blood flow can be observed in both fore- and hind-wings to almost their distal extremities; the forewing is extended laterally to the flying position in order to expose the underlying hindwing. Extension of either wing in this manner tends to reduce wing circulation. The blood can be observed to enter the body from the outstretched wings, to enter the mesatergal

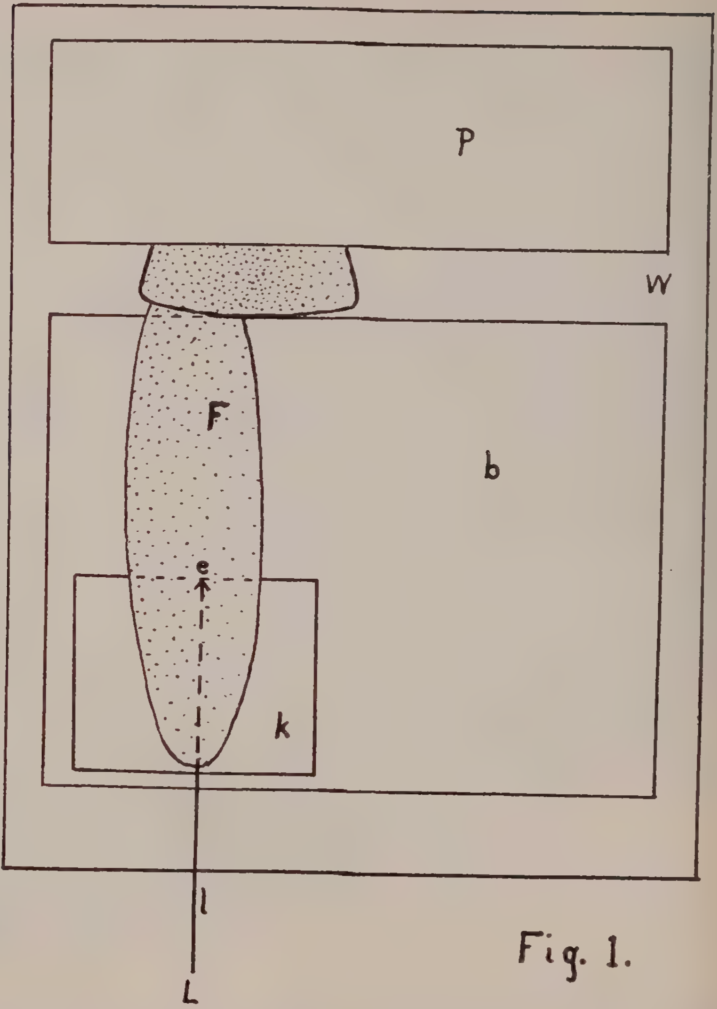

Fig. 1.

and metatergal pulsatile organs and to pass from these into the heart.

Wing circulation has also been detected by this method in *P. orientalis*, *P. fuliginosa*, and *Blatta germanica.*

6709

On Counting Mitotically Dividing Cells in the Blood of the Cockroach, Periplaneta orientalis (Linn.)*

J. FRANKLIN YEAGER AND OSCAR E. TAUBER.

From the Department of Zoology and Entomology, Iowa State College.

Although cell divisions in insect blood have been described by Hollande[1] and others, few quantitative counts apparently have been reported. This is a preliminary report of blood cell counts in *P. orientalis,* especially mitotically dividing cell (M.D.C.) counts. Large nymphs were used.

Method. Antennal blood is taken into a pipette, made to handle 1.22 mm.^{3} blood, and rapidly diluted; a total count is obtained with a hemocytometer. A small drop of the antennal blood is placed also in a larger drop of diluting fluid on a slide, immediately stirred to prevent cell coagulation, covered with a coverslip, and rimmed with oil to prevent evaporation. The cells are randomly counted (oil immersion) and the % M.D.C. determined. The diluting fluid consists of 0.081 M NaCl, 0.002 M KCl, 0.001 M $CaCl_2$, 0.005% Gentian Violet, 0.125% glacial acetic acid.

Results. Some of the counts obtained are in Table I. Groups I and II include normal animals. In Group I only anaphases and telophases, while in Group II prophases, metaphases, anaphases, and telophases were counted. Each animal was bled only once, to make the count. Group III contains 3 series of 3 animals each: 1 control, bled once for the count, and 2 experimental animals bled extensively at previous times; for example, No. A-2 was bled at 4 and again at 2 days before the count. Group IV includes 1 control, bled once for each count, and 1 experimental animal, also bled extensively after each count. This experiment lasted 14 days.

Discussion and Conclusions. The average total cell count of Groups I and II is 31,672 cells/mm.^{3} blood, or about the same as the count for *P. fuliginosa* obtained previously.[2] The average % M.D.C. is 0.07 in Group I and 0.51 in Group II. Assuming this difference to be due (1) to the counting of all phases in Group II and only anaphases and telophases in Group I and (2) to a longer

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* This report is part of work being done under a grant from the Rockefeller Fluid Research Fund, administered through Iowa State College.

[1] Hollande, A.-Ch., *Arch. Zool. exp. et gen.* (5 ser.), 1909, **2**, 271.

[2] Yeager, J. F., and Tauber, O. E., *Ann. Ent. Soc. Amer.*, 1932, **25**, 315.

TABLE I.
Cell Counts of Roach, *P. orientalis.*

Group	Animal No.	Duration of Exp. (days)	Total bl. cell count	M. D. C. per mm.^{3}	% M. D. C.	Condition
I	117C		14,400	29	0.20	Only A- and T-phases counted
	120C		47,186	22	0.05	” ” ” ” ”
	124C		57,600	9	0.02	” ” ” ” ”
	149C		24,894	51	0.21	” ” ” ” ”
	A-1		26,250	17	0.06	” ” ” ” ”
	B-1		40,310	11	0.03	” ” ” ” ”
	C-1		25,000	8	0.03	” ” ” ” ”
	D-1		26,450	6	0.02	” ” ” ” ”
	F-1		33,810	22	0.07	” ” ” ” ”
	H-1		55,280	11	0.02	” ” ” ” ”
		Ave.	35,118	18.5	0.071	
II	145		22,000	220	1.00	P-, M-, A- and T-phases counted
	146		30,250	88	0.29	” ” ” ” ”
	147		34,200	106	0.31	” ” ” ” ”
	148		26,450	119	0.45	” ” ” ” ”
		Ave.	28,225	133	0.51	
Tot. Ave.			31,672			0.51 ÷ 0.071 = 7.2
III	A-1		26,250	16.5	0.06	Control
	A-2		25,988	13.9	0.05	Bled 4, 2
	A-3		23,860	11.1	0.05	Bled 4, 2
	C-1		25,000	7.4	0.03	Control
	C-2		34,840	5.5	0.02	Bled 7, 5
	C-3		34,725	13.9	0.04	Bled 7, 5
	H-1		55,280	22.2	0.04	Control
	H-2		33,100	9.2	0.03	Bled 17, 15
	H-3		26,420	11.1	0.04	Bled 17, 15
IV	145	0	22,000	220.0	1.00	Normal
	”	1	10,650	69.2	0.65	1st bleeding on day 0
	”	3	9,225	35.1	0.38	2nd bleeding on day 1
	”	5	10,600	127.2	1.20	3rd bleeding on day 3
	”	7	8,995	139.4	1.55	4th bleeding on day 5
	”	10	6,605	49.5	0.75	5th bleeding on day 7
	”	12	10,150	154.0	1.52	6th bleeding on day 10
	”	14	(57,200)	(1790.4)	(3.13)	7th bleeding on day 12
	146	0	30,250	87.7	0.29	Normal
	”	1	28,450	335.7	1.18	Control
	”	3	26,090	60.0	0.23	”
	”	5	32,650	32.6	1.00	”
	”	7	36,550	175.4	0.48	”
	”	10	36,250	108.8	0.30	”
	”	12	44,300	168.3	0.38	”
	”	14	38,050	235.9	0.62	”

time duration of pro- plus meta- as compared to ana- plus telophase, then the observed difference would indicate that the average mitotically dividing cell remains in all mitotic phases about 7 times as long as in the last 2 phases.

Group III indicates that 1 or 2 previous hemorrhages may have no great effect on the % M.D.C. Group IV shows that when an animal is subjected to extensive (No. 145) or moderate (No. 146) recurring hemorrhages no great change occurs in the % M.D.C., except possibly at the end, when the animal becomes less active; and the total count decreases. On the 14th day, No. 145 was feebly active and hard to bleed, necessitating the use of blood from the leg; this may in part account for the high values then obtained. The facts that No. 145 bled freely on all days except the 14th and that its total count decreased indicate that each hemorrhage but the last was followed by a readjustment involving (1) a replacement of lost plasma by fluid and (2) a failure to replace all of the lost blood cells. No. 146, suffering slighter hemorrhages, seemed able to replace lost cells and fluid, maintaining its total count and always bleeding freely.

6710

Some Effects of X-Ray Irradiation on Respiration and Development.*

J. H. BODINE AND TITUS EVANS.

From the Zoological Laboratory, State University of Iowa.

In much of the previous work on the biological effects of X-rays morphological criteria alone have been used. It is, therefore, impossible to tell whether certain end-points are reached in similar or far different manner. This report states briefly the findings of a study of the effects of different intensities of irradiation on the oxygen consumption and development of the larva of the Mud-dauber Wasp *Sceliphron caementarium.*

The method used to separate latent-developing (diapause) larvæ from the developing organisms has been described.[1] Correlations between the morphological stages in development and the respiratory curve are also pointed out.

Four distinct morphological and physiological stages are distin-

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* Aided by grant from Committee on The Effects of Radiation on Organisms of National Research Council.

[1] *Biol. Bull.*, 1932, **63**, 235.

guished—larva, post-larva, pre-pupa, and pupa. When the diapause factor is present the organism remains normally in the quiescent larval stage for several weeks before developing. Such organisms when irradiated with 300-400 R. Units at the beginning of diapause remain in that condition several weeks longer than the controls. Higher intensities of irradiation (600-700 R. Units) shorten, and may even destroy the diapause factor, allowing the larva to develop at once. Still higher intensities (800-1500 R. Units) destroy diapause and permit of development which is subsequently inhibited.

Organisms in which the diapause factor is no longer present are accelerated by irradiation of 300-400 R. Units. The respiratory rate is higher and the time required to reach the pre-pupal stage is much shorter than in the controls. Six hundred to 700 R. Units inhibit, and 800-1000 R. Units are lethal before the pupal stage is reached.

Irradiation may affect (within limits) the physiological stage, active at the time of the treatment, without affecting following stages. If the physiological stage, dominant at the time of irradiation, is of such a character as to be antagonistic to development, then the response is not only quantitatively different from that of the developing stages, but will be apparently opposite in nature.

6711

Relationship Between Surface Activity of Homologous Carbamates and Effects on E.M.F. and Oxygen Consumption of Frog Skin.

EDGAR JOHN BOELL AND A. B. TAYLOR. (Introduced by J. H. Bodine.)

From the Zoological Laboratory, State University of Iowa.

The effects of different concentrations of methyl, ethyl, n-propyl, n-butyl, i-amyl, and phenyl carbamate in Ringer's solution on the electric potential of frog skin have been quantitatively and comparatively studied.

The skin of each frog used was divided into 11 pieces which were fastened over the mouths of tubes by means of rubber bands, and were bathed in Ringer's solution maintained at a temperature of 25° ± 0.5° C. Determinations of E.M.F. were made potentiometrically. After a preliminary period of approximately 2 hours in Ringer's solution, the skins were transferred to one containing a certain percentage of carbamate and were finally again placed in Ringer's.

Inasmuch as the magnitude of the electric potential of different pieces of skin, from the same or from different frogs, varied widely, but since the electromotive behavior of different skins was qualitatively similar, the E.M.F. in millivolts was transposed to per cent in order to permit of quantitative evaluation of the effect of carbamate. The potential at the time of application of carbamate (at which time electromotive equilibrium had been established) was arbitrarily designated as 100%. In terms of per cent, the skins from the same or from different frogs behaved quantitatively alike.

The normal E.M.F. of frog skin gradually decreases with time. The application of carbamate to skin causes a reversible diminution of E.M.F. which is a function of the duration of application and of concentration. The activity of the members of the homologous series conforms to the rule of Traube which states that the activity of the individuals of a series accords with the ratio 1- 3- 3^2- 3^3 etc.

The relative depression of E.M.F. by a carbamate parallels its action in lowering the surface tension of water. Stalagmometric and tensiometric measurements of the surface tension (air/water interface) were made at a temperature of 25° ± 0.2° C.

Measurements of the oxygen consumption of frog skin as influenced by carbamates were also made using the differential manometer. A diminution of oxygen consumption was found to accompany a decrease in potential. The per cent reduction of E.M.F., however, is generally greater than the per cent reduction of oxygen consumption.

Minnesota Section.

University of Minnesota, March 22, 1933.

6712

Non-effectiveness of Sodium Rhodanate in Antagonizing Morphine, Ether and Sodium Amytal.

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From the Department of Pharmacology, University of Minnesota.

Bancroft[1, 2, 3] and his collaborators have recently resurrected the old theory of Claude Bernard that narcotic and anesthetic drugs act by causing aggregation of the protein micelles in the cytoplasm. They correctly claim that if this is the correct theory the dispersing ions of the Hofmeister lyotropic series should antagonize this action. They have therefore administered NaSCN intravenously to rabbits and claim to have partly antagonized thereby the effects of morphine sulphate, ether, and sodium amytal. Their experimental series consisted of *one* treated rabbit and *one* control in each group. Disregarding entirely the well known variability of individual animals they have published very far reaching claims for the therapeutic efficacy of sodium thiocyanate (sodium rhodanate) for the treatment of morphine addiction, various forms of insanity and even general nervousness. Henderson[4] and Burkholder[5] independently repeated Bancroft's experiments on larger series of animals and were unable to confirm his results.

As the method described by Hirschfelder and Ridges[6] afforded us a very delicate technique for testing and following the course of

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[1] Bancroft, Wilder D., and Richter, George H., *J. Phys. Chem.*, 1931, **35**, 215.

[2] Bancroft, Wilder D., and Rutzler, J. E., Jr., *J. Phys. Chem.*, 1931, **35**, 1185, 3036.

[3] Bancroft, Wilder D., Gutsell, Robert S., and Rutzler, John E., Jr., *J. Phys. Chem.*, 1932, **36**, 1521, 2011.

[4] Henderson, V. E., and Lucas, G. H. W., *J. Pharm. and Exp. Therap.*, 1932, **44**, 253.

[5] Burkholder, Theodore M., *J. Lab. and Clin. Med.*, 1932, **18**, 29.

[6] Hirschfelder, A. D., and Ridges, A. H., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 958.

analgesia and narcosis we have used it in repeating Bancroft's experiments on a larger series of rabbits.*

Morphine Sulphate. Twenty-four rabbits were given doses of 25 to 50 mg. morphine sulphate per kilo subcutaneously. Three animals were given 150 to 500 mg. sodium rhodanate per kilo intravenously 5 to 10 minutes before the morphine, 11 animals received the same dose 20 to 30 minutes after the morphine. Ten controls received the same doses of morphine sulphate as the treated animals. Narcosis, i. e., the period during which the animals remained lying on their sides when placed in that position was a little longer in the rhodanate animals than in the controls, as was the time necessary before they recommenced coordinated movements, and also the time necessary for complete recovery. Curves of sensation tested in 4 rhodanate animals and 2 controls showed no constant difference between the two groups, except that analgesia lasted longer in the rhodanate animals.

Sodium Amytal. Eleven rabbits received 50 mg. Na Amytal subcutaneously and 6 of these 150 to 200 mg. Na rhodanate intravenously 45 minutes after the Amytal. As in the morphine series narcosis lasted a little longer (average duration 4 hours, 30 minutes) than in the controls (average duration 3 hours, 45 minutes). Five animals received 30 mg. Na Amytal, 3 of which received 150 mg. of Na rhodanate. Average duration of anesthesia was 2 hours, 30 minutes. Average duration of anesthesia of controls was 2 hours, 10 minutes.

Ether Anesthesia. In order to secure as nearly as possible uniform anesthesia of rhodanate animals and controls one treated animal and one control were placed simultaneously in a large etherizing chamber, a cylinder 70 cm. long x 30 cm. diameter into which a stream of air containing ether vapor, thoroughly mixed in baffle chambers was passed. A glass tube containing 2 copper wires inserted through the wall of the chamber was used for testing sensitivity at various stages of anesthesia. Eight rabbits received 150 to 200 mg. per kg. sodium rhodanate intravenously before the anesthetization. An equal number of controls were used.† Here also the rhodanate animals showed an average increased depth of anesthesia and retardation of recovery over the controls.

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* Since Prof. Bancroft has been awarded the Nichols medal of the New York Chemical Society for these researches, further investigation of his experimental data seemed necessary. Since Prof. Bancroft insists upon the use of Merck's Sodium Thiocyanate (Sodium Rhodanate), we used Merck's Sodium Thiocyanate recrystallized three times.

† After an interval of one week the control rabbit was given the NaSCN and the previous rhodanate animal was used as a control, thus ruling out individual variations.

These results confirm the work of Henderson and of Burkholder and are in absolute contradiction to the claims of Bancroft regarding the pharmacological action of sodium rhodanate. Bancroft's therapeutic claims based upon such experiments are therefore unwarranted.

6713

Thermolability of the Tissue Extract Factor in Extract Activation.

JOSEPH T. KING. (Introduced by F. H. Scott.)

From the Department of Physiology, University of Minnesota.

The writer has found that the addition of one volume of rabbit plasma to 60-100 volumes of chick embryo extract greatly increases the coagulative power of the extract[1]; that the increase of power is due to the appearance of a body which shows the same sensitivity to heparin as thrombin[2]; that the body reacts like thrombin in the presence and absence of ionic calcium[3]; that thermolability studies on the plasma factor indicate that this factor is prothrombin[4]. Mills and Matthews[5] found that mixtures of rabbit serum and lung extract show a temporary increase in coagulative power. Burns, Scharles, and Aitkin[6] have recently restudied the clotting power of extracts and sera from various sources. The papers cited above and some unpublished work made it reasonable to suppose that the cephalin in tissue extracts might be the factor involved in activation.

It is generally accepted that the only coagulation factor surviving boiling is cephalin. Mills[7] uses this as a method of distinguishing between tissue fibrinogen and cephalin in his study of blood platelets.

In this study chick embryo extract is made by extracting a 10 day embryo with Ringer solution. Coagulative power is tested by determining the shortening of the clotting time of a recalcified citrate rabbit plasma. Clotting time is done in 10 mm. tubes. In each test 2 drops of citrate plasma plus optimal amount 1% $CaCl_2$ plus 1 drop of the coagulant plus sufficient .9% NaCl to make a total of 10 drops are used. In this way the concentration of fibrinogen is kept constant. Activation is carried out by adding 1 part of citrate plasma to 20 parts chick extract. Pipettes are calibrated to drop 0.05 cc. Determinations have been made between 30° C. and boiling at intervals of 10°. Extracts are held at the designated temperature for 10 min.

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[1] King, Joseph T., *Arch. f. Exp. Zellforschung*, 1931, **10**, 467.

[2] King, Joseph T., *Am. J. Physiol.*, 1932, **101**, 64.

[3] King, Joseph T., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1112.

[4] King, Joseph T., in press.

[5] Mills, C. A., and Matthews, Stewart, *Am. J. Physiol.*, 1922, **60**, 193.

[6] Burns, E. L., Scharles, F. H., and Aitkin, L. F., *Am. J. Physiol.*, 1931, **97**, 233.

[7] Mills, C. A., *Chinese J. Physiol.*, 1927, **1**, 235.

There is a marked decrease in the power of the unactivated extract in this range, the main decrease taking place below 60° C. After testing, each extract is then activated and retested as soon as the added plasma coagulates. It is found that the increase in the clotting power of heated extracts is at least as great as in the unheated. In a typical experiment the following data were obtained: Recalcification time for the citrate plasma 14 min.; with 1 drop of unactivated, unheated tissue extract 4 min.; 1 drop same extract activated 45 seconds; 1 drop unactivated heated (boiling bath 10 min.) extract 11 min. 15 sec.; 1 drop same extract activated 1 min. 30 sec.

When the degree of activation is calculated according to the method used by Mills[5] it is seen that the heated extract is at least as capable of activation as the fresh unheated extract.

These data suggest that cephalin is the factor present in tissue extract which renders it capable of activation. This conclusion is in accord with facts developed by previous workers.

6714

Notes on Ultra-Violet Transmission of New and Used Dyed Cellophane Light Filters.

R. E. OLTMAN. (Introduced by G. O. Burr.)

From the Department of Botany, University of Minnesota.

Withrow[1] has described a series of dyed cellophane light filters for securing several sharp cut-offs in the ultra-violet region, and states that 2 sheets of cellophane soaked in sodium benzoate will absorb all radiations shorter than 2890 A.U., 2 sheets soaked in potassium acid phthalate absorbing all radiations shorter than 3130 A.U., and 3 sheets soaked in potassium cinnamate absorbing all radiations shorter than 3340 A.U. In a later publication, however, the originator of the filters[2] states that the sodium benzoate filter excludes all radiations shorter than 2970 A.U.

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[1] Withrow, R. B., *Bull. Bas. Sci. Res.*, 1931, **3**, 82.

To the investigator working with ultra-violet light, this discrepancy is important, since it means that 2 biologically active mercury lines, at 2894 and 2967 A.U., are either included or excluded in an irradiation, according to the cut-off of the filter. The author sent some new and used light filters that he had made up according to Withrow's directions to the DuPont Experimental Station at Buffalo, New York, and through their kindness was able to procure some very accurate transmission curves of both the new and used filters. It was found that the cut-off for the sodium benzoate filter was at 2890 A.U., but that there was an upward shifting of transmission amounting to 150 A.U. upon use, thus excluding 2 biologically active mercury lines. Summarizing the data obtained from the transmission curves of the filters: 1. Two thicknesses of a new sodium-benzoate soaked filter transmit the 2894 mercury line and exclude all shorter wave lengths; after more than 5 days of use this filter transmits only to 3024 A.U., thus excluding the extremely active 2894 and 2967 A.U. mercury lines. 2. Two thicknesses of a new potassium-acid-phthalate soaked filter transmit the 3132 A.U. mercury line and exclude all shorter radiations; there is no change in transmission in the filter in 5 days. 3. Three sheets of a new potassium cinnamate soaked filter transmit 3% of the energy of the 3132 A.U. mercury line, instead of entirely excluding all radiations shorter than 3340 A.U., as claimed by the originator of the filters. It transmits 38% of the 3342 A.U. mercury line, but since the 3132 line is much stronger than the 3342 line, 3% of the 3132 line is actually twice as much as 38% of the energy of the 3342 line. Thus the effect of this filter is much the same as that of the potassium acid phthalate filter; there is no shift in transmission upon use for 5 days.

It will be seen that in order to obtain the full effects of irradiation with a quartz-mercury lamp when using these filters to limit the wave length, it will be necessary to change the filters every 5 days in the case of the sodium benzoate filter, since the transmission of this filter shifts upwards at least 150 A.U. at that time. It is also seen that 3 sheets of a potassium cinnamate filter do not exclude all radiations shorter than 3340 A.U., as claimed by the originator of the filters, but also transmit the strong mercury line at 3132 A.U.

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[2] Withrow, R. B., and Benedict, H. M., *Bull. Bas. Sci. Res.*, 1931, **3**, 161.

6715

Effect of Ergotamine Tartrate on Blood Flow and Blood Pressure in the Femoral Artery of the Dog*

J. F. HERRICK. (Introduced by Hiram E. Essex.)

From the Division of Physics and Biophysical Research, The Mayo Clinic, Rochester, Minnesota.

In reviewing the literature[1, 2] on the pharmacodynamic activity and importance of ergot, it was found that its constituents, so far as the activity is concerned, may be divided into 2 groups: (1) specific alkaloids: ergotamine and ergotoxine, and (2) nonspecific amines: histamine and possibly tryramine. The specific alkaloids, to which I shall limit attention, are reported to produce a slow but persistent rise in blood pressure, diminishing with repeated dosage due to vasomotor paralysis until a condition is reached in which there is no rise, or else there is a fall which cannot be raised by epinephrine. Ergotoxine and ergotamine are pharmacodynamically identical. The researches of Brown,[3] and Barker[4] and their colleagues suggested the possibility that quantitative data on the actual changes in blood flow to the periphery brought about by this drug might be of some general interest. So far as I know, no such data are available.

The blood flow was measured by the thermo-stromuhr method developed by Rein.[5] The method permits quantitative observations under normal physiologic conditions in the intact animal. The femoral blood pressure was measured and recorded in the usual manner. All operative procedures were carried out under local anesthesia. The blood flow was measured in one femoral artery, and the blood pressure in the other femoral artery.

The dogs had been trained to lie quietly throughout all observations. After the normal blood flow and blood pressure were established, either 1.0 or 0.5 mg. of ergotamine tartrate (gynergen) was injected intravenously. The results of several experiments are given in the table. In 4 of the 6 experiments the blood flow was reduced to 25.5% of its normal value, on the average. The remaining 2 reductions were only 57 and 35%. There was a definite rise in the blood pressure, varying from 10 to 46 mm. Hg.

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* Work done under the direction of Dr. Hiram E. Essex at the Institute of Experimental Medicine, The Mayo Clinic.

[1] Thompson, M. R., *J. Am. Pharm.*, 1929, **18**, 1106; 1930, **19**, 11, 104, 221, 436, 705, 844.

[2] Barger, G., Ergot and Ergotism, 1931, pp. 151-160. Gurney and Jackson, London.

[3] Brown, G. E., quoted by Barker.

[4] Barker, N. W., *Med. Clin. N. Amer.*, in press.

[5] Rein, H., *Z. f. Biol.*, 1928, **87**, 394.

TABLE I.
Changes Caused by Ergotamine Tartrate.

Dog	Wt. kg.	Dose mg.	Blood Pressure mm. Hg. Before Injection	Blood Pressure mm. Hg. After Injection	Blood Flow, cc. per min. Before Injection	Blood Flow, cc. per min. After Injection
1	19.8	1.0	114	124	170	43
2	8.5	1.0			97	55
3	14.2	0.5	120	146	295*	70
4	16.8	0.5	120	166	160	56
5	14.4	0.5	114	150	86	23
6	21.5	0.5	120	160	81	21

* This large blood flow is due to experimentally induced hyperthyroidism.

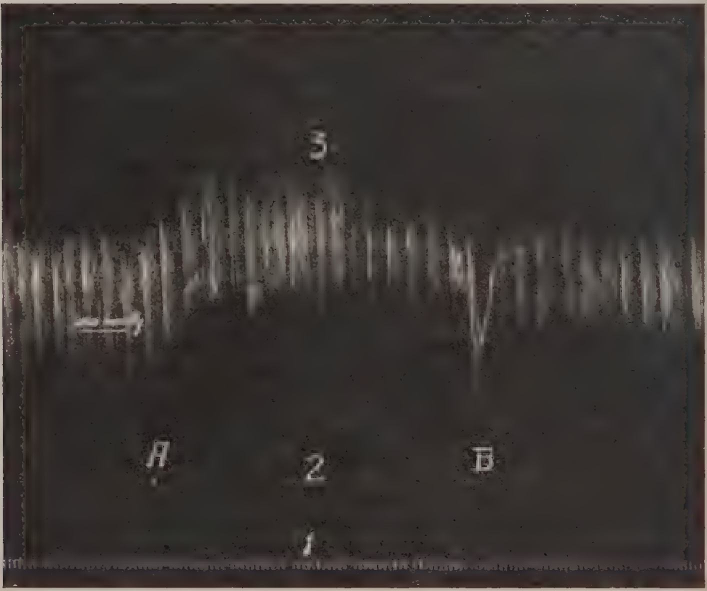

Fig. 1.

Emetic action of ergotamine tartrate as indicated by the blood pressure. *A* indicates injection (i.v.) of the drug. *B* indicates the first emesis occurring about 5 minutes after injection 1 = time in intervals of 5 seconds; 2 = 0 mm. of mercury; 3 = blood pressure.

The intravenous injection of ergotamine tartrate provoked prompt emesis in every instance, usually 5 minutes after the injection and several times thereafter. The emetic action of this drug has been reported previously.[6] Figure 1 shows the occurrence of this emesis about 5 minutes after the injection. This 5-minute interval between injection and emesis occurs with striking regularity in different dogs when the same dosage is used.

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[6] Koppanyi, T., and Evans, E. I., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1181.

Pacific Coast Section.

Mount Zion Hospital, San Francisco, March 15, 1933.

6716

Heterophile Ophthalmic Anaphylaxis.*

J. V. CHAMBERS. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University.

It would be of convenience to clinical allergists if conclusive experimental evidence were available in support of the postulated qualitative differences between synchronous, homologous, specific "antibodies" in different organs, tissues and body fluids of the same individual. Such differences are suggested by several recent laboratory studies, particularly by Cook and Spain's[1] alleged qualitatively different smooth muscle and cutaneous sensitizins in human serums, and by Schamburow's[2] alleged "reflex ocular immunity," specific agglutinins with the power of elective localization in the normal eye.

Seegal and Seegal[3] have recently demonstrated that a strictly local ophthalmic sensitivity can be produced in rabbits by the local injection of adequate sensitizing doses of routine antigens. This sensitivity is sufficient to give a specific unilateral ophthalmic allergy on intravenous injection with the same antigen. We have applied the Seegal technic to a study of the heterophile relationships between sheep erythrocytes and guinea pig kidney; doses, time intervals and allergic criteria being the same as those employed by them. Preliminary data from 35 rabbits suggest the following tentative conclusions:

(1) Practically all rabbits locally sensitized to sheep erythrocytes or to guinea pig kidney by the Seegal technic give marked unilateral

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* Supported in part by the Rockefeller Fluid Research Fund of the School of Medicine of Stanford University.

[1] Cook, R. A., and Spain, W. C., *J. Immunol.*, 1929, **17**, 295.

[2] Schamburow, D. A., *Z. f. Hyg. u. Infektionskrank.*, 1932, **114**, 456.

[3] Seegal, D., and Seegal, B. C., *J. Exp. Med.*, 1931, **54**, 249.

ophthalmic responses on intravenous injection with the homologous antigen. Approximately 75% of them give a demonstrable, though less marked allergic cross-reaction with the heterophile antigen.

(2) Anterior chamber injections of equivalent amounts of the alcohol-extractable lipoids from either antigen will not demonstrably sensitize the rabbit eye, nor is demonstrable sensitivity to either sheep erythrocytes or guinea pig kidney effected by local injection of the same lipoids adsorbed onto an alien protein "carrier" (*e. g.*, swine serum).

(3) Rabbits locally sensitized to either sheep erythrocytes or guinea pig kidney give no demonstrable ophthalmic response on intravenous injection with these lipoids or lipoid-protein complexes, nor does such injection demonstrate "desensitizing" the eye.

With the same natural antigens the alcohol-extractable lipoids are the dominant heterophile reacting factors in systemic (*i. e.*, humoral) anaphylaxis and test-tube complement-deviation reactions. From the above data, therefore, one would strongly suspect that there is a qualitatively different heterophile relationship in strictly local ophthalmic anaphylaxis, than there is in routine serum reactions.

6717

A Rapid Method for the Identification of Clostridium welchii.

ANSON HOYT. (Introduced by John F. Kessel.)

From the Department of Bacteriology, School of Medicine, University of Southern California, and the Laboratories of Unit I, Los Angeles County General Hospital.

In a study of the bacteriology of infected wounds, a rapid demonstration of the presence of *Cl. welchii* may be of considerable importance. Welch and Nuttall[1] showed that this organism produced abundant gas throughout the bodies of rabbits which had been injected intravenously with it, killed shortly thereafter, and incubated for a few hours at 37°C. The rabbit body provided an ideal anaerobic medium in which *Cl. welchii* would outgrow most aerobic organisms, and from which it could usually be isolated with ease.

White mice have been advantageously employed for the same purpose in this study. If a mouse is injected intravenously with a culture of *Cl. welchii*, killed within a few minutes, and incubated for 6 hours at 37° C., a very typical gross and microscopic picture is presented on autopsy. The tissues show a peculiar pink coloration. They impress one as being unusually dry and friable. The liver appears very pale, shrunken and dry, the surface is dull and the consistency is friable. The heart is also pale and friable, and usually contains no blood on section. The amount of gas production, although usually quite considerable, varies. Smears from the liver and from the interior of the heart show many large Gram-positive rods, with a morphology typical of *Cl. welchii*. These rods possess definite capsules when stained by the Hiss method.

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[1] Welch, W. H., and Nuttall, G. H. F., *Johns Hopkins Hosp. Bull.*, 1892, **3**, 81.

The above picture holds true even though the mouse is injected with a mixed culture containing relatively few *Cl. welchii*. In such a case the smears show some of the contaminating organisms, but they show, in addition, a relatively great number of Gram-positive capsulated rods, *i. e., Cl. welchii*.

This mouse injection method has recently been employed for bacteriologic diagnosis at the Los Angeles County General Hospital. An original smear was made from swabs sent in for diagnosis, and these swabs were then cultured in chopped veal infusion broth. If a moderate number of typical appearing Gram-positive rods were present in the original smear, ½ cc. of the culture was immediately injected intravenously into a mouse, and the presence or absence of *Cl. welchii* determined, on autopsy, 6 hours later. If a few or no Gram-positive rods were present in the original smear, the culture was incubated overnight, preferably, though not necessarily, in an anaerobic jar. If typical Gram-positive rods were present on subsequent smear, a mouse would be injected. In any event all suspicious Gram-positive rods were isolated and these organisms were classified according to their cultural characteristics and often, in addition, according to the lesions produced following guinea pig inoculation.

The above method has been utilized on 35 occasions. In 32 instances the results were very clear cut. *Cl. welchii* was shown to be present in 17 instances and absent on 15 occasions by the mouse method, and these results were subsequently confirmed by cultural procedures. Three mice yielded doubtful results.

6718

Combination of Certain Dyes with Deoxycholic Acid.

MABEL STOCKHOLM AND CARL L. A. SCHMIDT.

From the Division of Biochemistry, University of California Medical School, Berkeley.

The present experiments were undertaken in the attempt to throw some light on the mechanism whereby certain water soluble dyes such as rose bengal, when injected intravenously into animals, pass through the liver and are excreted in the bile.[1] Rose bengal in particular, on account of this property, has come into use as a test for liver function.[2] The experiments of Wieland and Sorge[3] and others[4] showing that deoxycholic acid combines with fatty acids, xylol, phenol, camphor, naphthaline, cholesterol and related substances to form the corresponding choleic acid and that excretion of the conjugated compound via the bile constitutes a method whereby the detoxicated product is eliminated, suggested that rose bengal might also combine with deoxycholic acid. The preparation of the rose bengal-deoxycholic acid complex was therefore undertaken.

Varying quantities of rose bengal were heated for several hours under a reflux condenser with known amounts of an alcoholic solution of deoxycholic acid (250 mg. of deoxycholic acid dissolved in 5-10 cc. of alcohol), an excess of dye always being present. The mixture was then concentrated *in vacuo* until a semi-viscous fluid was obtained. The presence of deoxycholic acid increases the solubility of the dye in alcohol. On addition of water the deoxycholic acid-dye compound was precipitated and the excess of dye was removed by repeatedly washing with water. The compound was recrystalized several times from alcohol containing a little rose bengal by addition of water. The excess of dye was removed by washing with water. The amount of dye contained in the complex was estimated colorimetrically by comparison with a standard solution of rose bengal containing 1 mg. in 10 cc. of alcohol. The dye could be dissociated from the compound by boiling with xylol.

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[1] Brakefield, J. L., and Schmidt, C. L. A., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 583.

[2] Delprat, G. D., *Archiv. Int. Med.*, 1923, **32**, 401. Delprat, G. D., Epstein, N. N., and Kerr, W. J., *Arch. Int. Med.*, 1924, **34**, 533. Kerr, W. J., Delprat, G. D., Epstein, N. N., and Dunievitz, M., *J. Am. Med. Assn.*, 1925, **85**, 942.

[3] Wieland, H., and Sorge, H., *Z. physiol. Chem.*, 1916, **97**, 1.

[4] Rheinboldt, H., *Ann. d. Chem.*, 1926, **451**, 256. Rheinboldt, H., König, D., and Flume, E., *Z. physiol. Chem.*, 1929, **184**, 219. Osten, F. W. R., and Grube, E., British Patent 287965, Dec. 30, 1926, quoted after *Chem. Abs.*, 1929, **23**, 484. Schönheimer, R., *Biochem. Z.*, 1924, **147**, 258. Schmidt, W., and Schmidt, C. L. A., *Univ. Calif. Pub. Physiol.*, 1930, **7**, 211.

TABLE I.

Amount of Dye Added		Dye found	Calculated	Proportion of dye to deoxycholic acid
mg.		%	%	
100	Tetrachlortetraiodo-fluorescein	20	19.9	1:10
75	" "	19.8		
60	" "	20.5		
50	" "	20.1		
75	Trichlortetraiodo-fluorescein	13.6	13.8	1:15
50	" "	14.7		
50	Tetrachlor-fluorescein	7.3	7.4	1:15

Table I gives the results obtained. The data indicate that the various rose bengal dyes form definite compounds with deoxycholic acid. Contrary to expectations no compound was formed when tetrachlorphthalein was similarly treated with deoxycholic acid.

Further evidence of compound formation between deoxycholic acid and rose bengal was obtained from conductivity estimations, carried out on solutions containing either 400 mg. of the complex or equivalents of deoxycholic acid, dye or mixtures of the latter. The specific conductivity data are: deoxycholic acid-rose bengal complex (400 mg. per 50 cc. of 95% alcohol), 7.75×10^{-6}; rose bengal (80 mg. per 50 cc. alcohol), 8.68×10^{-5}; deoxycholic acid (320 mg. per 50 cc. alcohol), 6.64×10^{-7}; mixture (80 mg. rose bengal + 320 mg. deoxycholic acid per 50 cc. alcohol), 8.47×10^{-5}.

Attempts to form similar compounds of deoxycholic acid with the fat soluble dyes, sudan III and scharlach R, which are eliminated in both bile and urine,[5, 6] gave negative results. These dyes could be almost completely removed by washing with ether subsequent to treatment with deoxycholic acid. The observations of Salant and Bengis[5] and of Joel and Schönheimer[6] point to the probability that the portion of these dyes which appears in the urine after administration is conjugated with glycuronic acid. The solubility of sudan III, as Joel and Schönheimer have pointed out, is markedly increased by the presence of sodium deoxycholate, a factor which may be concerned in the elimination of some of the dye in the bile.

We have observed when stearin choleic acid was treated with either sudan III or scharlach R in a manner similar to that used for the synthesis of the rose bengal-deoxycholic acid compound that it was not possible to wash the stearin choleic acid entirely free from dye by the use of ether. When the stearin choleic acid was decomposed by boiling with xylol, the dye was set free. The content of dye was about 1%. When stearin choleic acid was treated with rose bengal all of the dye could be removed by washing with water. The results suggest that possibly a compound was formed which contained both stearic acid and fat soluble dye.

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[5] Salant, W., and Bengis, R., *J. Biol. Chem.*, 1916, **27**, 403.

[6] Joel, E., and Schönheimer, R., *Centralbl. f. allgemein. Path.*, 1924, **34**, 625.

6719

Localizations of Poliomyelitic Virus During Incubation Period after Intranasal Instillation in Monkeys.*

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During the interval, corresponding with the incubation period in man, between intranasal inoculation of poliomyelitic virus in the monkey and the onset of spinal symptoms, the localizations of the virus are, with one exception, unknown. The exception is the olfactory bulb, where Flexner and Clark[1] found virus 48 hours after application to the nasal mucosa. At that time none was demonstrable in the medulla or spinal cord. In the present study, *Macacus rhesus* monkeys were given 3 intranasal instillations, without trauma, of active MV virus at intervals of 3 hours, or within a total period of about 6 hours. Monkeys so treated were sacrificed on the third, fourth, fifth, sixth, and seventh days, respectively. Brain and spinal cord were removed under aseptic precautions. Specimens were removed from various parts of the central nervous system, ground and suspended in physiological salt solution to approximate 10% concentration and injected intracerebrally in monkeys in 1.5-2.0 cc. amounts. These animals were observed for the appearance of the usual signs of poliomyelitis and, if positive, were examined postmortem for gross and histological lesions.

The efficacy of the intranasal method of inoculation employed was shown by a series of 26 monkeys used in a separate study (unpublished) by Schultz and Gebhardt, in which 23, or 88%, devel-

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* This study was supported in large part by a grant from the Rockefeller Fluid Research Fund of the School of Medicine of Stanford University.

[1] Flexner, S., and Clark, P. F., PROC. SOC. EXP. BIOL. AND MED., 1912, **10**, 1.

oped typical poliomyelitis. The average incubation period in these was 8.2 days (minimum, 7 days; maximum, 10 days).

The results follow:

Three days' incubation: no virus in left olfactory bulb, left thalamus, medulla, cervical and lumbar cord (pooled), cervical and lumbar intervertebral ganglia (pooled). (6 monkeys.)

Four days' incubation: virus in left olfactory bulb; no virus in hippocampus, left hypothalamus, medulla, cervical and lumbar cord and intervertebral ganglia (pooled). (6 monkeys.)

Five days' incubation: virus in left olfactory bulb, right olfactory bulb, left hypothalamus, and medulla; no virus in hippocampus, thalamus, midbrain, cerebellum, or cervical cord. (10 monkeys.)

Six days' incubation: virus in left olfactory bulb, left hypothalamus, left thalamus, right thalamus, midbrain, medulla; no virus in right olfactory bulb, hippocampus, precentral gyrus, postcentral gyrus, right hypothalamus, caudate nucleus, cerebellum, cervical cord, lumbar cord, cervical and lumbar intervertebral ganglia. (17 monkeys.)

Seven days' incubation: virus in left olfactory bulb, right olfactory bulb, hippocampus, right thalamus, midbrain, medulla, cervical cord (anterior half), cervical cord (posterior half), lumbar cord (anterior half), cervical and lumbar cord (posterior half), left intervertebral ganglia, right intervertebral ganglia; no virus in precentral and postcentral gyri (pooled), left hypothalamus, right hypothalamus, left thalamus, cerebellum, lumbosacral segment of cord. (19 monkeys.)

The experiments show a gradual advance of the infection, apparently by continuity via connecting nerve tracts, through the central nervous system during the incubation period. The pathway of invasion extends from the portal of entry in the nasal mucosa and olfactory nerves to the olfactory bulb and thence down the brainstem to the spinal cord. The cord is not invaded until the seventh day. Late in the incubation period some areas in the upper brainstem that were previously infected no longer show virus in demonstrable amounts.

6720

Effect on Ovarian Weight of Prolonged Administration of Anterior Lobe Extract.*

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From the Department of Obstetrics and Gynecology, Stanford University School of Medicine.

It was shown that the administration of a pregnancy blood extract at daily doses which produce comparatively small increases in ovarian weight (100 to 200%) in 5-day experiments, results in very marked enlargement of the ovaries if the injections are continued for from 2 to 3 weeks.[1]

These studies have been extended to include the effects induced by an extract prepared from dried sheep anterior hypophyses by the method of Wallen-Lawrence and Van Dyke.[2] This procedure is essentially the same as that used for preparing the pregnancy blood extracts and consists in extracting with a sodium acetate-acetic-acid buffer, pH 4.2 to 4.5, and reprecipitating with 95% alcohol.

A total of 78 rats have been treated for periods up to 20 days in duration and with various doses of the extracts. The experiments were begun in animals 20 to 23 days of age, and each rat was injected twice daily.

It has been observed that when immature female rats are given daily injections of sheep anterior pituitary extract in such doses that the ovaries increase from 100 to 500% in weight in the first 5 days, the continued daily administration of the same dose for periods up to 20 days fails to produce any further marked increase in ovarian weight. For instance, in one series of 21 rats each animal was given 0.5 cc. of a comparatively weak extract daily and groups of 5 or 6 were sacrificed every 5 days. The average percentage increase in ovarian weight over controls on the 5th day was 420% (body weight 33 gm., ovaries .060 gm.). On the 10th day it was 375% (body weight 47 gm., ovaries .054 gm.); on the 15th it was 462% (body weight 60 gm., ovaries .078 gm.), and on the 20th it was 412% (body weight 63 gm., ovaries .073 gm.).

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* Supported by a grant from the Rockefeller Fluid Research Fund of Stanford University School of Medicine. The sheep anterior pituitary glands were supplied by Messrs. Parke, Davis & Company, through the courtesy of Dr. E. A. Sharp.

[1] Fluhmann, C. F., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 149.

[2] Wallen-Lawrence, Z., and Van Dyke, H. B., *J. Pharm. and Exp. Therap.*, 1931, **43**, 93.

It would seem that these results are in marked contrast to those produced by the ovary-stimulating hormone found in human pregnancy blood, and add additional evidence in support of the conception that these hormones are not identical. On the other hand, it is possible that the extract of sheep anterior lobe contains a thyreokinetic hormone and that an effect on the thyroid may account for some of the differences in ovarian response, as shown in guinea pigs for acid extracts of cattle hypophyses by Loeb and Friedman.[3]

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[3] Loeb, L., and Friedman, H., PROC. SOC. EXP. BIOL. AND MED., 1931, **26**, 172.

Peiping Section.

Peiping Union Medical College, February 22, 1933.

6721

Experimental Reproduction of Joint Sprains.

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The object of the work was to study the histological changes in the soft structures and bone at varying periods of time after experimentally produced joint sprain and to determine the rate of healing.

Six rabbits were used in the development of the method. Varying amounts of manual force were used on both ankle joints or both knee joints. The animals were killed immediately afterward and the extent of injury to the soft structures was determined by gross examination. In this way it was discovered how to produce a simple sprain without fracture. Sprains were produced thus on 12 rabbits. From 1 to 6 weeks later, the animals were sacrificed and autopsied. The joints were removed and examined grossly and microscopically.

One week after the sprain, there was definite swelling of the joints, especially marked at the site of injury. There, the synovial membrane was thickened and oedematous with a few dilated blood vessels near its surface. The joint fluid was increased in amount, more viscid and yellow than normal. There was slight pannus formation at the osteo-cartilaginous junctions on the injured side of the joint. The subcutaneous tissue and the loose connective tissue of the mesotendons showed evidence of old hemorrhage. There was no blood within any of the tendon sheaths. Microscopic sections showed hemorrhage into and under the synovial membrane. The synovial cells had marked vacuolization. The subsynovial tissue was edematous, with capillary congestion, early fibroblastic proliferation and considerable leukocytic and lymphoid cell infiltration. In places the surface of the synovial membrane was covered with fibrin, while in others the fibrin was seen in the subsynovial tissues. Near the attachments to the bone, the injured capsule and ligaments showed similar changes consisting of oedema, fibroblastic proliferation and lymphoid-cell infiltration. The loose connective tissue beneath the synovial lining of the tendon sheaths presented a picture essentially the same as that seen in the joint synovia.

At 2, and 3 weeks there were still definite signs of acute inflammation of the synovial tissue as evidenced by swelling and by leucocytic and lymphoid-cell infiltration. There was great increase of the fibroblasts in the subsynovial tissue. Hemosiderin pigment also was found in large amounts in the phagocytic cells of the inflamed area. At this stage hyalinized masses of fibrin were seen within the synovial tissue and on the synovial surface. The tendon sheaths and the soft tissue surrounding the capsule and ligaments showed changes similar to those which were found after 1 week, except that there was more marked fibroblastic proliferation.

After 4 weeks, there was no external evidence of swelling or other signs of old injury. Microscopic examination of the tendon sheaths, synovial tissue, and ligaments still showed evidence of old hemorrhage. The number of fibroblasts was decreased, but the number of intercellular collagen fibers was increased. Many small capillaries were seen in this young connective tissue. In certain places the synovial membrane showed small foci of degeneration. There was still considerable oedema of the loose connective tissue but the infiltration with leukocytes and lymphoid cells was much less than that shown in the sections taken after 3 weeks.

Microscopic examination at 6 weeks showed complete healing of the traumatized parts. There was a late stage of fibrosis with shrinkage and contraction of the connective tissue structures.

6722

Phenolphthalein Starch Medium for Rapid Isolation of V. Cholerae.

ALBERT C. H. YEN. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College.

Among the various media that have been advocated for rapid isolation of *V. cholerae,* the most widely used is the alkaline peptone enrichment medium which, however, is by no means perfect. During a cholera epidemic in Peiping in the summer of 1932, we felt that a more efficient medium was desirable. To meet this demand the following medium (phenolphthalein-starch solution) was devised, utilizing the unique property of rapid fermentation of starch by *V. cholerae*[1] in a highly alkaline solution in combination with an indicator as an index for its growth. As the cholera epidemic was over when the technique of preparation of this medium had been perfected, its efficiency was tested out on stools seeded with *V. cholerae* and gave satisfactory results.

The medium is prepared by dissolving peptone (Witte) 2 gm., maltose 1 gm., potassium nitrate 0.5 gm., sodium carbonate (crystallized) 0.5 gm., sodium chloride 10 gm., and magnesium chloride (crystallized) 0.5 gm. in 900 cc. of distilled water. The solution is boiled for 3 minutes and filtered. 100 cc. of a 5% solution of soluble starch previously boiled for 2 minutes is added to 900 cc. of the above solution and mixed thoroughly. The mixture is filtered through cotton and then through asbestos filter, yielding a clear filtrate which is first measured and then sterilized by boiling for 3 minutes. (The solution cannot be autoclaved in the ordinary way as the starch is decomposed by prolonged heating). The sterilized filtrate is kept in ice chest. Just before use the reaction of the solution is adjusted to pH 9.0-9.2 in the following way: First 5 cc. of the indicator phenolphthalein (saturated solution in 50% alcohol) is added to each litre of the filtrate and the solution is carefully brought to decolorization point (pH 8.3) by addition of necessary amount of N/20 HCl or N/20 NaOH and finally to each litre of the decolorized solution is added exactly 1.5 cc. of N/I NaOH which brings the solution to pH 9.0-9.2 (pink colored). The titrated solution is distributed into separate sterile flasks in 50 cc. lots, ready for seeding. (The solution after titration for pH should be used fresh.)

For cultivation of *V. cholerae* from stool, 0.1-0.2 cc. of the liquid stool or a loopful of the solid stool is inoculated into 50 cc. of the P.S. medium (Phenolphthalein starch medium) and incubated at 37°C. for 5-8 hours. The decolorization of the solution which indicates growth of cholera or cholera-like vibrio is watched. The microscopic or macroscopic agglutination test can then be made from the surface growth of the decolorized solution with known anti-cholera serum. If decolorization is incomplete by the end of 8 hours' incubation or the growth is too thin for agglutination tests,

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[1] Gordon, M. H., *Centralb. f. Bakt.*, 1906, **1**, 42.

1 cc. from the seeded P.S. medium is transferred to a fresh flask of P. S. medium and incubated for another 5-8 hours.

As a preliminary test for the efficiency of the P.S. medium a comparison of this medium with the A.P. medium (alkaline peptone enrichment medium[2]) is made. From each of the 30 specimens of non-choleraic stools 2 stool emulsions are made. (A) containing 2 loopfuls of stool and 0.5 cc. 18 hours' peptone water culture of *V. cholerae* in 5 cc. saline, and (B) with the addition of 0.5 cc. cholera-phage. From the individual stool emulsions, 0.2 cc. is inoculated separately into 50 cc. lots of the P.S. medium and the A.P. medium for both series and incubated at 37° C. for 6 hours. At the end of this period plating on C.R. medium (China-blue-rosalic-acid agar pH 7.6) and macroscopic agglutination tests from each flask of the P.S. and A.P. media are made.

1. *Agglutination tests.* From 30 specimens of the treated stools, the P.S. medium gave positive agglutination test for *V. cholerae* in 30 specimens or 100% in A. series and in 10 specimens or 33.3% in B. series; while the A.P. medium gave 20 specimens or 66.7% in A. series and 1 specimen or 3.3% in B. series. Thus, the P.S. medium gave a higher percentage of positive agglutination tests than that obtained from the A.P. medium whether cholera-phage was present or not. It is also shown that the presence of cholera-phage in stools containing *V. cholerae*, greatly reduces the percentage of positive isolations.

2. *Plate growths.* The percentage of specimens giving growth of *V. cholerae* and *B. coli* in C.R. plate from the A.P. and P.S. media is given in Table I, which shows that *V. cholerae* grows more easily in the P.S. medium by the end of 6 hours' incubation, and *B. coli* is somewhat inhibited in the P.S. medium.

TABLE I.

% of Positive Plate Isolation of *V. cholerae* from Alkaline Peptone and Phenolphthalein Starch Media.

Types of Colonies	A. Series Stool + *V. cholerae* Alkaline peptone	 Phenolph. starch	B. Series Stool + *V. cholerae* + choleraphage Alkaline peptone	 Phenolphthalein starch
	%	%	%	%
V. cholera	60	86.7	13.3	30
B. coli	96.7	66.7	96.7	73

The above results suggest that for the rapid isolation and diagnosis of *V. cholerae* from stools, the phenolphthalein starch medium is more satisfactory than the alkaline peptone enrichment medium, even in the presence of cholera-phage. Hence in convalescent cholera cases when cholera-phage has appeared in the stool, the use of phenolphthalein starch medium would be a distinct advantage. Furthermore, the presence of the indicator serves not only for titration of the medium to a desired alkalinity but also as a growth index of the culture. Although its value must not be over-estimated until data from clinical cases of cholera are available, it seems worth while to record here the favorable results obtained under experimental conditions with the phenolphthalein starch medium and to recommend its trial in cholera cases.

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[2] Bengstone, I. A., *Hyg. Lab. Bull.*, 1924, **139**, 37.

6723

Rough Forms of Vibrio Cholerae from Convalescents.

W. K. CHEN. (Introduced by C. E. Lim.)

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It has been observed[1] that dissociation of *Vibrio cholerae* could be enforced by bacteriophage *in vitro* and the resulting variants showed morphological and serological changes from the parent strain. Pasricha and his coworkers[2] reported that by subjecting a freshly-isolated phage-resistant cholera strain to repeated cultivation on laboratory media they could obtain certain colonies showing morphological, cultural and serological variation. They also produced evidence suggesting that the loss of agglutinability induced by a high titered cholera serum might occur in cholera vibrios normally during the course of the disease.

During the summer of 1932 a fairly widespread epidemic of cholera extended to Peiping where a limited number of cases not treated with bacteriophage was available for study. The purpose of the present investigation was to make a careful study of culturally-positive cases from the beginning to the end of the disease so as to isolate rough forms of *Vibrio cholerae* and cholera bacteriophage from the stool and vomitus specimens obtained on different days of the disease. As soon as a specimen reached the laboratory a few cc. were introduced into peptone water medium (pH 8.4) and incubated at 37°C. for 2-4 hours. Then plates of China-blue-rosalic-acid medium were inoculated and the peptone water cultures were filtered through Berkefeld N candles for detection of bacteriophage. If the suspicious colonies on the plates were numerous or more than 10, only 10 single colonies were picked up and inoculated on peptone water; otherwise all the suspicious colonies were picked for culture on various sugar media. Tests were carried out with every typical cholera strain isolated for its motility in semi-solid medium and checked by the hanging drop method, for "smoothness" or "roughness" by Millon's reaction,[3] for agglutinability with known freshly prepared high titered serum from characteristic pure smooth strains, and for susceptibility to stock cholera phage[4] and to the patient's own phage if it happened to be present in the filtrate of the stool cultures during convalescence. For detection of phage in the filtrate, the liquid culture method[5] was used against a known pure smooth culture freshly isolated from a fatal dose and also against the patient's own primary smooth culture which was isolated from the first stool specimen after admission. Cases if diagnosed culturally after admission as cholera were followed to the end of the disease; daily specimens of fresh stool (in the acute stage even 2-3 stools per day) and vomitus were examined. The cholera colonies isolated from 195 stool and 16 vomitus specimens out of 21 positive cases were divisible into 3 distinct types according to their "smooth" or "rough" reactions, susceptibility to cholera-phage and agglutinability with high titered serum. Smooth type, lysible colonies by cholera-phage with full titer agglutination was always isolated in abundance during early acute stage of the disease when cholera-phage had not yet appeared. Among the 21 cases there were 13 such patients admitted early enough to give positive pure cultures of smooth type cholera vibrio during the first to third day of the disease.

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[1] Chen, W. K., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1160.

[2] Pasricha, C. L., de Monte, A. J., and Gupta, S. K., *Ind. Med. Gaz.*, 1932, **67**, 64.

As soon as cholera-phage appeared in the stool and the patient was convalescent, smooth type colonies became scanty or entirely absent, and intermediate and rough resistant variants of comparatively low titer agglutination (1:160-1:640) against known high titered serum took their place. Among the 21 cases the convalescence of 18 patients could be followed. From practically every convalescent case the rough type of resistant variants was isolated at the same time with the appearance of cholera-phage. Motility of the rough variants was also sometimes impaired and non-motile colonies of rough cholera vibrio were isolated in 4 cases. The motility, however, could be restored on subsequent transfers in laboratory media. The rough type of cholera vibrio besides giving different degrees of "roughness" to Millon's reagent· also showed granular growth in broth, spontaneous agglutination in saline and sometimes rough morphology of surface as well as deep colonies on ordinary laboratory media. From the convalescent cases few colonies of absolutely non-agglutinable ("NAg" type) cholera-like vibrios appeared in 5 cases out of the 18 recovered patients. These "NAg" strains of cholera-like vibrious were rough to Millon's reagent, resistant to our stock cholera-phage and also to freshly isolated cholera-phage mixtures from convalescent cases. It is also of interest to point out here that in severe cases of cholera-phage appeared comparatively later than that in mild cases which also gave earlier cultures of predominating rough type cholera vibrio. Cholera-phage in the stool lasted much longer after recovery than did the cholera variants. Cultures of the stool from convalescent cases were repeatedly negative for cholera variants but cholera-phage was isolated up to the time of each patient's discharge. Cholera-phage was not found in any specimen of the vomitus although cholera vibrio was positive in one of the 16 specimens. This is to be expected since patients usually had vomiting only during the acute stage of the disease when phage had not yet developed.

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[3] Gautier, R., League of Nations, Health Section Eastern Bureau: Singapore, 1930. (Note on Bacteriophage.)

[4] Morison, J., and Vardon, A. C., *Ind. J. Med. Res.*, 1929, **17**, 48.

[5] d'Herelle, F., Malone, R. H., and Lahiri, M. N., *Ind. J. Med. Res. Mem.*, 1930, No. 14.

To determine the serological relationship of the rough colonies anti-sera were prepared by immunizing rabbits with each of the following strains: 4 strains of smooth type cholera vibrio ("Ag"), 4 strains of slightly agglutinable rough variants and 4 non-agglutinable cholera-like vibrios ("NAg"). Cross agglutination and absorption tests among the "Ag" and "NAg" forms brought out the antigenic independency of the "Ag" type of true cholera vibrios from the "NAg" forms of cholera-like vibrios. Anti-sera of the 4 strains of "NAg" cholera-like vibrios which did not constitute a serological homogeneous group were antigenically different from each other in contrast to the "Ag" strains of true cholera vibrios which were antigenically similar. Rough resistant variants (some non-motile) which appeared at the same time during convalescence with the "NAg" cholera-like vibrios were agglutinable only to a low titer (1:160-1:640) against the true cholera serum and had absolutely no agglutination with any anti-serum of the 4 "NAg" cholera-like vibrios. Anti-sera prepared from the non-motile, slightly agglutinable rough variants gave a low titer (1:640) agglutination only, but could agglutinate each other and the smooth cholera vibrios to the full titer but not the "NAg" cholera-like vibrios. The above serological reactions do not suggest that the slightly agglutinable variants are intermediate types between the "Ag" type of true cholera vibrio and the "NAg" types of cholera-like vibrios. The "NAg" cholera-like vibrios may be "mutants" of true cholera vibrio possibly resulting from phage action *in vivo*. But the question has to be considered also in the light of the "NAg" cholera-like vibrios being of no etiological significance and mixed with the rough variants of true cholera vibrio during convalescence when the activity of the true smooth cholera vibrio is checked by cholera-phage.

To determine whether the variants are reversible, all the non-motile rough variants of true cholera vibrio and the "NAg" cholera-like vibrios were subjected to daily repeated transfers in peptone water medium (pH 8.4) for 90 days. Only the rough variants of true cholera vibrios showed a tendency to revert to better agglutinable "S" or "SR" motile forms. "NAg" cholera-like vibrios remained stable as to their in-agglutinability against the true cholera serum. Animal passages also failed to have any effect upon the agglutinability of the "NAg" cholera-like vibrios. Reversion by bacteriophagic action as described by Pasricha had been tried, but as all the "NAg" cholera-like vibrios were absolutely resistant to all our cholera-phage mixtures isolated so far, no positive findings have as yet been obtained.

Conclusions. Within a limited number of 21 cholera cases proven by positive cultures carefully followed up from the beginning to the end of the disease, it has been found that dissociation of cholera vibrio as determined by its chemical, morphological and serological changes was found to occur naturally during convalescence with the development of bacteriophage against the pure smooth cholera cultures.

Occasional non-agglutinable strains of cholera-like vibrios mixed with the slightly agglutinable rough variants of true cholera vibrio have also been isolated during convalescence. But serological reactions do not bring the "NAg" forms into any relationship with either the pure smooth type or the slightly agglutinable rough variants of true cholera vibrio. The "NAg" cholera-like vibrios are not reversible and at present the writer has no means to conclude that they are positively the mutants of true cholera vibrio.

On the other hand, the slightly agglutinable rough variants, including the non-motile ones, have a weak somatic antigen in common with the true smooth cholera vibrio. They are reversible and are actually the true rough variants of cholera vibrio apparently resulting from the bacteriophage-action during the natural course of the disease.

New York Meeting.

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6724

A Pandemic of Rabbit-Pox.

HARRY S. N. GREENE. (Introduced by L. Pearce.)

From the Laboratories of The Rockefeller Institute for Medical Research.

A recent outbreak of a highly contagious disease in our breeding colony produced such serious consequences that it seems desirable to direct attention to the infection. This report concerns the disease produced by natural transmission. The experimental infection and the causative agent have been studied by Pearce, Rosahn, and Hu.[1]

The first cases of the disease appeared in December, 1932, and within a month every animal in a colony of 1500 rabbits had been infected.

Clinically, the disease resembled small-pox in man. It was characterized by an elevation of temperature with a pock-like eruption of variable extent in the skin and mucous membranes and an incubation period of from 5 to 10 days. In typical cases, the first sign of infection was the appearance of erythematous spots; papules were then formed in these areas and later became umbilicated and covered by crusts. These lesions varied in size from minute points to nodules a centimeter in diameter; they occurred singly, in groups, or as confluent masses with extensive edema and in some cases were hemorrhagic. The papules occurred over the entire body but were most numerous in the ears, the eyelids and brows, the lips, the nape of the neck, the skin of the trunk, and the scrotum.

The mouth, nose, and pharynx were frequently the seat of large nodules, diffuse infiltrations, and ulcerative lesions which, in extreme cases, obstructed respiration and prevented eating. Such animals usually showed a mucopurulent discharge with the formation of brownish crusts about the nose and lips suggestive of snuffles or a clear blood stained secretion from the nose.

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[1] Pearce, L., Rosahn, P. D., and Hu, C. K., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 894.

Eye lesions were of frequent occurrence and in some animals were the only visible manifestation of infection. The lymphoid system was invariably affected and one of the earliest signs of infection was enlargement and induration of the popliteal nodes. In males, the testicles usually showed a nodular or diffuse orchitis with edema of the scrotum.

Animals with severe infections showed obvious signs and symptoms of illness. Upper respiratory and buccal infections were accompanied by labored respiration, mouth breathing, and in protracted cases, by emaciation and weakness. Mild cases of infection were, however, asymptomatic and the existence of infection could be determined only by the most careful examination. Moreover, animals with extensive lesions often appeared to be in perfect health.

The course of the disease was variable. Death frequently occurred within a few hours after the first signs of infection were noted or after a period of days or weeks. Many animals recovered after severe infection, visible lesions healing with the formation of scars. The mortality rate varied with breed, sex, age, and physiological status. Among animals under 2 weeks of age, the mortality approximated 100%, but the rate decreased with age until, in normal adults, the mean mortality was less than 20%. In certain breeds, as the Himalayan, there were no adult deaths, while in others, as the Belgian, the breeding stock was decimated. Abortions were frequent.

The findings at autopsy were also variable and not always characteristic. The organs most consistently affected were the liver, spleen, and lungs.

In typical cases, the liver was enlarged, pale yellow or yellowish grey in color, firm in consistency, and showed a profusion of small, opaque grey or pearly white nodules. These were usually absent in fulminating cases. In some animals, the general appearance of the organ was not greatly altered and the focal lesions formed the most definite evidence of disease.

The spleen was enlarged, dark red in color, and firm, and usually showed focal lesions comparable with those in the liver.

The lungs were occasionally normal but as a rule showed either small focal lesions, areas of consolidation, or a lobar pneumonia. In exceptional instances, the entire lungs showed an acute, diffuse pneumonic process with clearly defined pearly grey or white focal lesions distributed throughout.

Focal or diffuse lesions were found in many other organs and tissues such as the adrenals, ovaries, salivary glands, lymph nodes, bone marrow, subcutaneous tissues, fat, muscle, and periosteum.

Histologically, lesions showed a central area of necrosis with a surrounding zone of edema or hemorrhage and mononuclear cell infiltration. Inclusion bodies were not found.

In conclusion, it should be noted that during the terminal stages of the epidemic, the severity of the infection diminished greatly so that a large proportion of animals of the most susceptible age groups survived infections contracted at this time.

6725

Experimental Transmission of Rabbit-Pox by a Filterable Virus.

LOUISE PEARCE, PAUL D. ROSAHN AND C. K. HU.

From the Laboratories of The Rockefeller Institute for Medical Research.

Experimental transmission of the spontaneous rabbit-pox discussed in the preceding communication[1] was attempted early in the course of the pandemic. Since the result of bacteriological examinations together with the clinical picture of the condition made it appear unlikely that we were dealing with any of the usual bacterial agents, the initial experiments were carried out with Berkefeld "V" filtrates of various organs and tissues from rabbits showing well marked symptoms of the disease. In other experiments, unfiltered suspensions were used. The organs employed included: testicle, liver, spleen, lung, popliteal lymph nodes, brain, spinal cord, blood, and skin. Injections were made into the testicles of rabbits procured from outside sources.

The results of the first experiments were positive in that an orchitis and scrotal edema were pronounced within 48 hours, fever developed (105°-107°), the animals appeared ill, frequently a diarrhea was present, and death occurred in from 4 to 8 days. This condition has been produced successively from rabbit to rabbit by means of filtered material (usually testicle) and the agent is now in the fifteenth serial passage. Other routes of inoculation have also been successfully employed, namely, intracutaneous, subcutaneous, intravenous, intraperitoneal, intramuscular, intracerebral, and nasal and conjunctival instillation. By using small doses and certain routes of inoculation, it has been possible to reproduce all the clinical and pathological features of the spontaneous disease including the characteristic pock eruption of the skin and mucous membranes. In addition, complete recovery has occurred in these circumstances.

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[1] Greene, H. S. N., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 892.

The agent is active in tissue stored in 50% glycerol at ice box temperature for at least 94 days. Unfiltered material is much more potent than that filtered through a Berkefeld "V" candle but an "N" filtrate is also active. Very small doses are sufficient to induce pronounced lesions in various sites and death has followed the intratesticular injection of 1 cc. of filtrate in a dilution of 1:8000.

Rabbits which have recovered from either the spontaneous or the experimental disease have so far been refractory to reinoculation. Furthermore, the serum from recovered animals has the property of neutralizing the virus in a 1:1 serum-virus mixture as shown by intracutaneous tests.

The disease does not resemble Virus III or infectious myxoma of rabbits either clinically or pathologically, and the results of crossed inoculation experiments and of virucidal tests show that there is no relationship between the respective agents. Similar experiments with rabbits immune to the Shope tumor and to the Shope tumor plus infectious myxoma are now in progress.*

With respect to vaccine virus, however, there is evidently some relationship but the two viruses are apparently not identical. Rabbits immune to the N. Y. City Board of Health vaccine virus are refractory to injections of rabbit-pox filtrates variously applied but intradermal injection of unfiltered pox virus results in a positive although highly modified skin reaction. Adult vaccine virus immune rabbits do not develop rabbit pox when exposed to "open" cases but young vaccine virus immunes do. In these instances, the disease was considerably milder than that developed by normal control rabbits (litter mates). The results of virucidal tests with vaccine virus immune serum and rabbit pox virus are in keeping with the above observations in that the serum has a definite but only a partial protective power. We have not been able to produce lesions typical of vaccinia in either the cornea or scarified skin but in several instances cytoplasmic inclusions indistinguishable from Guarnieri bodies were found in corneal epithelial cells. These observations, together with the distinctive clinical features of the spontaneous and experimental rabbit pox disease, tend to the conclusion that the present agent is not identical with the vaccine virus with which we have worked.

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* We are indebted to Dr. T. M. Rivers for the infectious myxoma experiments and to Dr. Carl TenBroeck for those with the Shope tumor.

Information on the host range of the virus is still incomplete. We have not succeeded in inoculating rats but in one instance the virus has been recovered from the testicle of a guinea pig inoculated intratesticularly. The inoculation of mice has also been accomplished by means of the intracerebral route. With this procedure death occurs in 4 to 6 days and the brain contains active virus. It is now in the fifth serial mouse to mouse passage. Other animal species and the calf in particular, are being tested for susceptibility to the virus.

6726

Cultivation of Vesicular Stomatitis Virus.

H. R. COX, J. T. SYVERTON AND P. K. OLITSKY.

From the Laboratories of The Rockefeller Institute for Medical Research, New York.

Carrel, Olitsky, and Long[1] reported the successful cultivation of the virus of vesicular stomatitis of horses in 1928. In their cultivation experiments, guinea pig tissues were used throughout. The method they employed consisted in placing embryonic tissue or adult bone marrow in contact with the virus which had been suspended in Tyrode's solution for 1½ to 24 hours and then transferring to Carrel flasks containing coagulated plasma to which serum was added when marrow was used, or embryonic juice when embryonic tissue was used. The flasks were incubated for 7 to 10 days at 39°C.

No further reports of successful *in vitro* cultivation of the virus of vesicular stomatitis have been made.

This paper describes a much simplified method, similar to that employed by Li and Rivers[2, 3] in the cutlivation of vaccine virus, for the cultivation of the virus of vesicular stomatitis. A satisfactory and readily available technique for the titration of the virus is given. Through the kindness of Dr. W. E. Cotton, samples were sent us of Indiana and New Jersey strains of virus which had been propagated in guinea pig pads for several years. The respective strains were then carried through 25 or more pad passages, filtered through Seitz filters, and this filtrate, or the Seitz filtrate of infected mouse brain tissue,[4] was used to initiate the cultures. The tissue employed was that of a 9 to 12 day chicken embryo which, after removal of the head and limbs, was minced in 3.5 cc. of Tyrode's solution. The Tyrode's solution was freshly prepared every 10 days according to the following formula: NaCl, 8.0 gm.; KCl, 0.2 gm.; $CaCl_2$, 0.2 gm.; $MgCl_2$, 0.1 gm.; NaH_2PO_4, 0.05 gm.; $NaHCO_3$, 0.5 gm.; glucose, 1.0 gm.; double distilled water q.s.ad. 1000.0 cc.; pH adjusted to 7.7 and sterilized by filtration through a Seitz or a tested Berkefeld N filter. Rivers' flasks[2] were used throughout.

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[1] Carrel, A., Olitsky, P. K., and Long, P. H., *Compt. rend. Soc. biol.*, 1928, **98**, 827.

[2] Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465.

[3] Rivers, T. M., *J. Exp. Med.*, 1931, **54**, 453.

The cultures were prepared by diluting one part of the titrated Seitz filtrate with 9 parts of Tyrode's solution. To 3.5 cc. of the diluted suspension contained in a Rivers' flask were added 3 drops of the minced embryonic tissue suspension. New cultures were subsequently prepared by directly diluting the old culture 1:10 with Tyrode's solution and to 3.5 cc. of the resultant diluted material 3 drops of a freshly prepared suspension of minced chick embryonic tissue were added. All cultures were incubated at 37.5°C. for 3 days and bacteriological controls were made for each successive generation. This simple method suffices for the propagation of an indefinite number of culture generations.

In a preceding report,[4] it was shown that the virus of vesicular stomatitis was uniformly lethal for white mice 36 to 72 hours after the intracerebral inoculation* of 0.03 cc. of an active virus suspension. This neurotropism for the white mouse provides a readily available, satisfactory, and inexpensive means of titration. Titrations were carried out in dilutions of 10^3 to 10^8 with every third generation.

Both strains of virus were used in the initiation of the 5 series of cultures made.

Series A. A Seitz filtrate of the Indiana strain derived from mouse brain tissue, lethal for mice in a dilution of 1-10,000 or 10^4, was used. This was carried through 18 generations. Titration of the third generation was lethal in a dilution of 10^6; of the sixth 10^6; of the ninth 10^6; of the twelfth 10^5; of the fifteenth 10^5; and of the eighteenth 10^5.

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[4] Cox, H. R., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 654.

* Ether anesthesia was used in all operative procedures.

Series B. A Seitz filtrate of the New Jersey strain derived from guinea pig pad tissue, lethal in a dilution of 10^3, was used. This has been carried through 12 generations to the present. Titration of the third generation was lethal in a dilution of 10^5; of the sixth 10^4; of the ninth 10^5; and of the twelfth 10^4.

Series C. A Seitz filtrate of the New Jersey strain derived from guinea pig pad tissue, lethal in a dilution of 10^2, was used. This has been carried through 15 generations to the present. Titration of the third generation was lethal in a dilution of 10^5; of the sixth 10^4; of the ninth 10^5; of the twelfth 10^5; and of the fifteenth 10^6.

Series D. A Seitz filtrate of the Indiana strain derived from mouse brain tissue, lethal in a dilution of 10^3, was used. This has been carried through 15 generations to the present. Titration of the third generation was lethal in a dilution of 10^6; of the sixth 10^6; of the ninth 10^5; of the twelfth 10^5; and of the fifteenth 10^5.

Series E. A Seitz filtrate of the Indiana strain derived from guinea pig pad tissue, lethal in a dilution of 10^1, was used. This was carried through 15 generations. Titration of the third generation was lethal in a dilution of 10^3; of the sixth 10^5; of the ninth 10^5; of the twelfth 10^6; and of the fifteenth 10^6.

The final generation in each passage series gave rise to typical lesions in the pads of guinea pigs.

In conclusion, it appears that the virus of vesicular stomatitis is capable of multiplication *in vitro* by the simple method of cultivation described, and that the white mouse provides a satisfactory means of titration.

The close generic relationship of the virus of vesicular stomatitis to the incitant of foot-and-mouth disease suggests that the virus of foot-and-mouth disease may be cultivated with equal facility.

6727

Isolation of Treponema Pallidum from a Subcutaneous Sarcoid.

CHESTER N. FRAZIER AND CH'UAN-K'UEI HU.

From the Division of Dermatology and Syphilology, Department of Medicine, Peiping Union Medical College.

Various hypotheses have been advanced in regard to the etiology of the several types of cutaneous and subcutaneous tumors passing under the name of sarcoids.[1, 2, 3, 4] Upon the basis of histologic structure it seems most likely that the cutaneous type as described by Boeck,[5] and the subcutaneous type of Darier and Roussy[6] belong to the general group of infectious granulomas. Although the common assumption favors their tuberculous origin, it has been only rarely that acid-fast bacilli have been demonstrated directly in stained tissue sections,[7, 8] and animal inoculations, as a rule, have yielded only negative results.[9, 10] Recently, however, acid-fast bacilli have been found in the tracheo-bronchial lymph nodes of guinea pigs, inoculated with tissue from sarcoid, in which no gross lesions of the viscera of the animals were detected.[11]

Sarcoids are also known to occur infrequently in patients with frank syphilitic infection. In such cases the histologic appearance of the tumors is like that usually considered typical of sarcoid. No reference to the isolation of any bacterial organism from any of the syphilitic cases has been found although Stillians[12] searched for acid-fast bacilli and *Treponema pallidum* in stained tissue preparations from the patient he observed. The fact that sarcoids in patients with syphilis respond promptly and disappear under antisyphilitic therapy has little, if any weight as an argument for their syphilitic nature. Similar lesions in patients presumably free of syphilis respond at times in an equally prompt manner.

Inoculation of animals susceptible to syphilis as well as those to tuberculosis offers a more hopeful method of study than has been heretofore pursued. This method was employed in studying a case of sarcoid in a syphilitic patient seen in the Peiping Union Medical College. *Treponema pallidum* was isolated from a rabbit inoculated with the sarcoid tissue, but no acid-fast bacilli were demonstrated in the tissues examined from 2 guinea pigs inoculated with the same material.

The patient was a Chinese man 42 years of age. For one year

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[1] Pautrier, L. M., *Ann. de dermat. et syph.*, 1914, **5**, 344.

[2] Kuznitzky, E., and Bittorf, A., *München. med. Wchnschr.*, 1915, **62**, 1349.

[3] Schaumann, J., *Acta derm-venereol.*, 1921, **2**, 409.

[4] Motta, J., *Ann. de dermat. et syph.*, 1931, **2**, 1180.

[5] Boeck, C., *J. Cutan. Dis.*, 1899, **17**, 543.

[6] Darier, J., and Roussy, J., *Ann. de dermat. et syph.*, 1904, **5**, 144.

[7] Kyrle, J., *Arch. f. Dermat. u. Syph.*, 1921, **131**, 33.

[8] Dittrich, O., *Dermat. Z.*, 1931, **60**, 395.

[9] Volk, R., *Wien. klin. Wchnschr.*, 1913, **26**, 1425.

[10] Goeckerman, W. H., *Arch. Dermat. and Syph.*, 1928, **18**, 237.

[11] Ravaut, P., Valtis, J., and Nelis, P., *Compt. rend. Soc. de biol.*, 1929, **101**, 444.

[12] Stillians, A. W., *J. Am. Med. Assn.*, 1921, **77**, 1615.

there had been a slowly growing, painless tumor of the subcutaneous tissues of the forehead, about 2x4 cm. in size, slightly elevated above the skin and firmly indurated. Under the impression that the tumor was a fibrosarcoma, it was excised. A histological diagnosis of tuberculosis of muscular tissue was made. After a period of 2 years the patient was seen again with a recurrence of the tumor in and around the old scar, and also with a similar tumor of the right temporal region, which had been present for 8 months. This was indurated, about 3.5x4 cm. in size, and composed of 3 confluent plaque-like nodules apparently involving the deeper part of the skin, the subcutaneous tissues and muscle. The lungs were essentially normal on roentgenological examination. The tuberculin test was positive. The patient gave a history of venereal exposure and a genital chancre 8 years before admission; the blood Wassermann and Kahn reactions were positive. Part of the lesion of the right temporal region was excised for histological study and animal inoculation. Microscopically, the epidermis and upper corium were about normal. There was an increase in fibrous tissue in the deeper part of the corium, and the hypoderm had a lobulated appearance due to the presence of well-defined groups of epitheloid cells separated by rather dense fibrous tissue septa. At the periphery of the nests of epitheloid cells was a zone of round cell infiltration and fibroblasts. An occasional giant cell was found in these cellular areas. No acid-fast bacilli or spirochetes were seen in the stained tissue sections.

Under continuous treatment with neoarsphenamin and a bismuth salt there was complete involution of the remaining lesions. At the end of a 3-year period during which there had been no recurrence of the tumors, the serological tests for syphilis were negative.

A part of the sarcoid removed from the temporal region was finely emulsified in sterile normal salt solution. This suspension was used for inoculation; 0.5 cc. was injected into the left testis of 2 normal rabbits, and an equal quantity was injected into the peritoneal cavity of 2 normal guinea pigs. One rabbit developed an orchitis 51 days after inoculation, and the blood Wassermann and Kahn reactions became positive. *Treponema pallidum* was demonstrated in the material aspirated from the testicular lesion. No metastatic lesions were observed during the 4 months the animal was under observation. Spirochetes were not seen in the material aspirated from the testis of the second rabbit which developed an orchitis 16 days after inoculation. On 2 occasions during the period of orchitis the blood Wassermann and Kahn tests were negative.

During the 4 months the rabbit was observed no metastatic lesions were detected.

The 2 guinea pigs remained vigorous and healthy for 4½ months after inoculation. At the end of this time there was no evidence of disease in the viscera or serous membranes of the 2 animals. No acid-fast organisms were seen in stained tissue preparations from the lungs, spleen, liver and inguinal lymph node in one animal, and from the spleen in the other.

This single observation supports the opinion that sarcoids in syphilitic patients are syphilitic in origin. Although a blood-stream infection was not eliminated as a possible source of the organisms recovered from the animal developing syphilis, its occurrence in an infection of 8 years standing is unlikely. It seems reasonable to assume that although the tumors described by Boeck and by Darier and Roussy belong to a more or less distinct clinical group, they do not represent a common etiologic entity.

6728

Exophthalmos in Thyroidectomized Guinea Pigs by Thyrotropic Substance of Anterior Pituitary, and the Mechanism Involved.

DAVID MARINE AND S. H. ROSEN.*†

From the Montefiore Hospital, New York City.

We pointed out[1] that chronic, progressive, bilateral exophthalmos, produced in immature (2-3 months old) rabbits, maintained on a diet of alfalfa hay and oats, by the daily intramuscular injection of 0.05-0.1 cc. methyl cyanide, occurs in association with thyroid insufficiency (that is, (a) marked hyperplasia of the thyroid or (b) thyroidectomy). It is obvious, therefore, that exophthalmos is not dependent upon either a normal or abnormal thyroid secretion. Neither do the medulla and cortex of the suprarenal gland have any specific or initial relation to exophthalmos.

To obtain further information on the nature of exophthalmos we have produced it in young normal and thyroidectomized guinea pigs by using acetic acid extracts of anterior pituitary powder (Armour) that Spaul,[2] Uhlenhuth and Schwartzbach,[3] and Loeb and Bassett[4] have shown to contain the thyrotropic hormone and with which Loeb and Bassett have produced exophthalmos in guinea pigs. The data are given in the following table.

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* Fellow of Montefiore Hospital.

† Aided by a grant from the Ella Sachs Plotz Foundation.

[1] Marine, D., Rosen, S. H., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 649.

TABLE I.

Guinea Pig No.	Thyroid intact Wt.	Age	Sex	Amount thyrotropic‡ hormone injected intraper. daily	Examination for Exophthalmos by Days 2nd	4th	6th	8th	10th	12th	14th
	gm.	days		cc.							
1	276	28	M	2	—	—	+?	+?	+	+	+
3	241	16	M	2	—	+	+	+±	+	+	+
9	206	16	F	2	—	—?	+±	+	+	+	+
10	175	16	M	2	—	—	—	—	—	—	—
13	184	19	M	1 cc. for 10 days then 2 cc.	—	+	+	+	+	+	+±
14	240	24	M	”	—	—?	—?	+	—?	+?	+
	Thyroidectomy										
5	159	16	F	2	—	+	+?	+±	+±	+±	+
6	184	16	M	2	—	+	+	+	+?	+	+
7	141	16	F	2	—	—?	+	+±	+	+	+
8	160	16	M	2	—	—?	—?	—	+?	+	—?
11	213	19	F	1 cc. for 10 days then 2 cc.	—	—	+?	—	—	—?	+
12	187	19	M	”	—	—?	—?	+?	—	—?	+±
15	169	19	M	”	—	—	—	—	—	—?	—

‡ Prepared after the method of Loeb and Bassett.

It is clear that acetic acid extracts of ox anterior pituitary powder produce exophthalmos as well in thyroidectomized guinea pigs as in those with intact thyroids. Also, as in the case of methyl cyanide, the exophthalmos does not appear in guinea pigs with intact thyroids until they have developed thyroid hyperplasia (thyroid insufficiency), and to both cyanide and anterior pituitary extract some animals are resistant. Since both thyroidectomy and thyroid hyperplasia cause hypertrophy of the anterior pituitary, and since both methyl cyanide and the thyrotropic factor of the anterior pituitary cause exophthalmos in the absence of the thyroid, it is probable that the exophthalmos caused by methyl cyanide is due to its stimulating effect on the anterior pituitary which in turn stimulates the centers in the mid-brain controlling the sympathetic innervation of the eye (the pupillo-dilator muscle and the muscle of Müller). It also appears probable that cyanides act indirectly to cause thyroid hyperplasia by stimulating the anterior pituitary to an increased production of the thyrotropic hormone. Whether the stimulation of the anterior pituitary is a direct cyanide action or an indirect result of stimulation of the autonomic nerve centers in the hypothalamus caused by the general tissue oxidation inhibiting effect of cyanides is unknown, although there is abundant evidence in favor of the latter view: that is, that the thyroid response, the pituitary response, the adrenal response, and the exophthalmos are the expressions of the organism's attempt to overcome the metabolism depressing action of HCN.

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[2] Spaul, E. A., *Brit. J. Exp. Biol.*, 1924, **2**, 33.

[3] Uhlenhuth, E., and Schwartzbach, S., *Brit. J. Exp. Biol.*, 1927, **5**, 1.

[4] Loeb, L., and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 490.

Our present view of the mechanisms involved in the production of thyroid hyperplasia and of exophthalmos by cyanides and by the thyrotropic hormone may be presented schematically as follows:

Inhibition of tissue oxidations (cyanide)

Hypothalamic centers

Anterior pituitary **Chromaffin system**
(Thyrotropic factor)

Thyroid **Hypothalamic centers**
(Sympathetic)

Exophthalmos

Cyanides appear to act by inhibiting metabolic processes. This inhibition stimulates the hypothalamic centers, which in turn stimulate the pituitary to produce the thyrotropic hormone, which in turn powerfully stimulates the thyroid gland and independently the sympathetic centers in the mid-brain causing exophthalmos.

6729

Respiratory Quotient of Cerebral Cortex in B_1 Avitaminosis.

H. E. HIMWICH, J. F. FAZIKAS, N. RAKIETEN AND B. SANDERS.

From the Laboratory of Physiology, Yale University, School of Medicine.

The investigation of Peters and his coworkers[1] has shown that the ability of the brain to oxidize lactic acid is decreased in polyneuritic pigeons. Since the respiratory quotient of the cerebral cortex of normal animals is unity,[2] probably due to the oxidation of

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[1] Gavrilescu, N., Meiklejohn, A. P., Passmore, R., and Peters, R. A., *Proc. Roy. Soc.*, 1932, **110 B**, 431.

[2] Himwich, H. E., and Nahum, L. H., *Am. J. Physiol.*, 1932, **101**, 446. Dickens, F., and Simer, F., *Biochem. J.*, 1930, **24**, 1301.

TABLE I.
Respiratory Metabolism of Cerebral Cortex.

Animal	$\frac{CO_2 \text{ mm}^3/\text{mgm/hr}}{O_2 \text{ mm}^3/\text{mgm/hr}}$	R. Q. of excised cortex	R. Q. of brain *in situ*
Pigeon No. 1	$\frac{0.68}{0.89}$	0.76	
Pigeon No. 2	$\frac{0.88}{1.02}$	0.86	
Pigeon No. 3	$\frac{0.63}{0.96}$	0.66	
Dog	$\frac{1.13}{1.30}$	0.87	0.89
	$\frac{1.10}{1.33}$	0.83	
	$\frac{1.06}{1.39}$	0.76	

lactic acid.[3] the determination of the respiratory quotient of the cortex during B_1 avitaminosis is of importance.

Three pigeons and one dog were fed on diets deficient in vitamin B_1 and were studied after they showed definite signs of polyneuritis. The respiratory metabolism of the cerebral cortex of the pigeons was examined in the Warburg apparatus. The respiratory quotient of the brain of the dog was determined in 2 ways: (1) directly from the arterial-venous difference of CO_2 and O_2, and (2) the brain was then excised and studied in the Warburg apparatus. The results presented in Table I indicate respiratory quotients below unity probably caused by a diminished ability of the brain to oxidize lactic acid.

6730

Effect of Methylene Blue and Cyanide on Respiration of Cerebral Cortex, Testicle, Liver and Kidney.

H. E. HIMWICH, J. F. FAZIKAS AND M. H. HURLBURT.

From the Laboratory of Physiology, Yale University, School of Medicine.

The respiratory metabolism of tissues of various organs was studied in the Warburg respiration apparatus. The tissues were suspended in a phosphate medium buffered at pH 7.4 with lactate as the substrate. Three different samples of methylene blue were used and, with each, a diminution of O_2 uptake was observed in 47 experiments on the cerebral cortex of rat, dog and cat. Whenever methylene blue was added to the solution the CO_2 production of the surviving brain tissue was decreased to a greater extent than the O_2 consumption; the respiratory quotient therefore fell.

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[3] Holmes, E. G., *Biochem. J.*, 1930, **24**, 914.

Cyanide, on the other hand, caused a diminution in the O_2 uptake of excised cortex in each of 66 observations. The decrease in CO_2 output was usually similar for equal concentrations of cyanide and varied inversely with the concentration of cyanide. However, the decrease in O_2 consumption varied from observation to observation even if the concentration of cyanide was kept the same. Since the O_2 uptake varied, the respiratory quotient after treatment with cyanide fluctuated.

Even though cyanide and methylene blue separately decreased O_2 uptake, in the presence of both substances the oxygen consumption tended to return to the normal value. The fact that the CO_2 consumption is so small is evidence that the oxidations activated by methylene blue do not proceed in the usual manner. A characteristic experiment is presented in the table (Exp. 1).

The results of 6 observations of the testicle are similar to those found on the brain (Exp. 2).

TABLE I.
Effects of Methylene Blue and Cyanide on Respiratory Metabolism of Excised Organs (mm³/mgm/hr).

Exp. No.	Organ	Control		0.005% MB		0.006% NaCN		MB + NaCN	
		CO_2/O_2	R.Q.	CO_2/O_2	R.Q.	CO_2/O_2	R.Q.	CO_2/O_2	R.Q.
1.	Cortex of brain	$\frac{0.86}{0.85}$	1.01	$\frac{0.28}{0.52}$	0.54	$\frac{0.26}{0.27}$	0.97	$\frac{0.14}{0.76}$	0.19
2.	Testicle	$\frac{0.47}{0.49}$	0.96	$\frac{0.15}{0.30}$	0.50	$\frac{0.13}{0.15}$	0.87	$\frac{0.12}{0.28}$	0.43
3.	Kidney	$\frac{2.77}{3.47}$	0.80	$\frac{1.20}{1.67}$	0.75	$\frac{0.22}{0.58}$	0.38	$\frac{0.34}{0.71}$	0.48
4.	Liver	$\frac{0.77}{1.15}$	0.66	$\frac{0.49}{0.97}$	0.51	$\frac{0.32}{0.42}$	0.76	$\frac{0.39}{0.87}$	0.45
5.	Liver	$\frac{0.84}{1.19}$	0.70	$\frac{1.25}{1.74}$	0.72	$\frac{0.43}{0.85}$	0.50	$\frac{0.72}{1.33}$	.054

In 16 experiments on the kidney, methylene blue produced a diminished oxygen consumption without any constant effects on the R.Q. Similarly cyanide decreased the oxygen uptake, but, in those experiments in which methylene blue was added, this was raised again toward the normal value (Exp. 3).

The effect of methylene blue on oxidations in the liver varied in different experiments. Five times, oxygen consumption decreased; once no change was observed and 5 times oxygen uptake increased. Cyanide, however, always diminished respiration and the diminution was reversed by methylene blue (Exps. 4 and 5).

6731

Effects of Methylene Blue on Respiratory Metabolism of the Rat.

H. E. HIMWICH AND W. GOLDFARB.

From the Laboratory of Physiology, Yale University, School of Medicine.

Methylene blue exerts definite effects in the metabolism of excised tissue.[1] It is therefore of importance to determine the action of methylene blue in the intact animal. The respiratory metabolism of 6 rats was studied by means of a Haldane open circuit apparatus after various periods of fasting. Then 0.5 cc. M/100 methylene

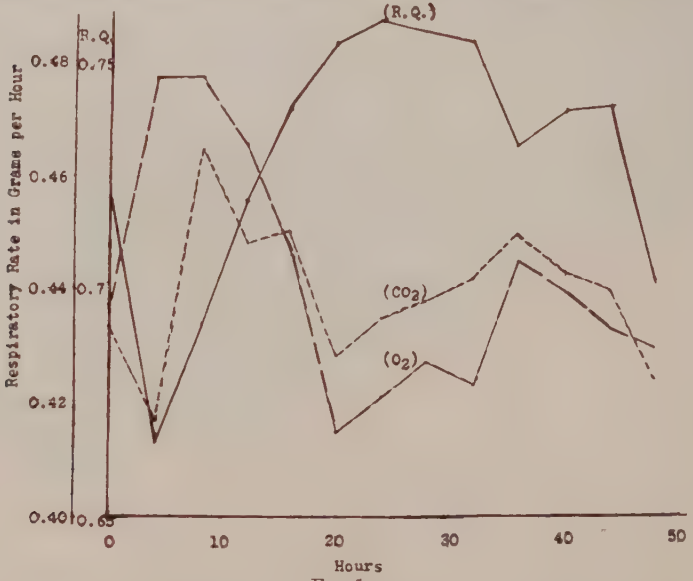

FIG. 1.
Effect of Methylene Blue. Composite of 4 experiments.

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[1] Himwich, H. E., Fazikas, J. F., and Hurlburt, M. H., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 904.

blue per 100 gm. of body weight was injected intraperitoneally. The CO_2 production and O_2 consumption were measured for varying periods during the following 48 hours. The results in all 6 animals bear a striking similarity. The composite graph depicts the observations of the 4 animals that were in the postabsorptive condition.

The graph discloses that there is a fall in the R.Q. which is followed by a subsequent rise. The early fall in the quotient is caused in part by a diminished production of CO_2 and partly by an increased consumption of O_2. The greater O_2 uptake indicates that the decreased production of CO_2 occurs despite an increased pulmonary ventilation. The fall of the R.Q. carries it to a value below 0.7 but the total R.Q. for the entire period of 48 hours in these 4 rats varied between 0.711 and 0.737.

6732

Effect of Methylene Blue on the R.Q. of the Brain in Situ.

H. E. HIMWICH, N. RAKIETEN, L. H. NAHUM, D. DU BOIS AND R. SANDERS.

From the Laboratory of Physiology, Yale University, School of Medicine.

TABLE I.
Effect of Methylene Blue.

	R.Q. of brain	pH of arterial blood	Rectal temp. C.°	Sp. Gr.
Amytal	0.99	7.24	39.3	1.0653
MB 15 min.	0.77	7.25	39.8	1.0659
1 hr.	0.92	7.26	40.2	1.0673
1¾ hr.	0.54	7.27	40.7	1.0698

An early effect of methylene blue in the intact animal is to decrease the R.Q.[1] Methylene blue exerts a similar action on the R.Q. of excised cortical tissue of the brain[2] causing a decrease below the physiological value of unity.[3] This communication presents the results of preliminary experiments of the changes produced by methylene blue on the R.Q. of the brain *in situ*.

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[1] Himwich, H. E., and Goldfarb, W., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 906.

[2] Himwich, H. E., Fazikas, J. F., and Hurlburt, M. H., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 904.

[3] Himwich, H. E., and Nahum, L. H., *Am. J. Physiol.*, 1932, **101**, 446. Dickens, F., and Simer, F., *Biochem. J.*, 1930, **24**, 1301.

Ten cc. of 1% solution of methylene blue were injected into the common carotid artery of 4 dogs under amytal anesthesia. Blood samples from the femoral artery and the superior longitudinal sinus were analyzed for CO_2 and O_2, pH and specific gravity. The respiration and rectal temperature of the dogs were recorded.

The arterial-venous differences of CO_2 and O_2 indicated that the R.Q. of the brain diminished after the injection of methylene blue. At the same time the rectal temperature of the animals rose. This is in agreement with the greater O_2 uptake noted previously.[1] The specific gravity of the blood increased as did the pulmonary ventilation, the latter producing a rise of the pH of the arterial blood. The results of a characteristic experiment are presented in tabular form.

6733

Studies on Bacterium Granulosis in Relation to Trachoma. (III-IV).*

CHARLES WEISS.†

From the Oscar Johnson Institute, Washington University, St. Louis, Missouri.

By repeated instillation of *freshly isolated* cultures of *Bacterium granulosis*, or by a single subconjunctival injection of this organism, granular conjunctivitis was produced in several rhesus monkeys. This confirms the work of Noguchi,[1] Olitsky, Knutti and Tyler,[2] and Thygeson.[3] It was observed, moreover, that by repeated irritation with powdered, dry, sterilized Portland cement (applied by means of a cotton swab), or by previous inoculation with a virulent hemoglobinophilic bacillus *(H. influenzae)*, the monkeys' conjunctivae were at times rendered more vulnerable to infection with *B. granulosis*. Best results were, however, obtained by the subconjunctival injection of freshly excised, macerated, infected monkey tissue (tarsus and conjunctival follicles) containing *B. granulosis*.

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* For previous publications in this series, see Weiss, C., *J. Inf. Dis.*, 1930, **47**, 107, and PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 398.

† Present address: Research Laboratories of Mount Zion Hospital, and Hooper Foundation of the University of California. Conducted under a grant from the Commonwealth Fund.

[1] Noguchi, H., *J. Exp. Med.*, 1928, **48**, Suppl. 2.

[2] Olitsky, P., Knutti, R., and Tyler, R., *J. Exp. Med.*, 1931, **54**, 31; and subsequent papers in the *J. Exp. Med.*

[3] Thygeson, P., *Am. J. Ophth.*, 1932, **15**, 293.

Clinically and histologically, the conjunctival lesions thus induced were similar to those produced by transfer of human tissue; that is, bits of conjunctiva, follicles, or secretions which had been *freshly removed* from patients with active trachoma, including pannus. Lesions produced by the latter method (which consisted of either subconjunctival injection or rubbing the material thoroughly into the conjunctiva with the aid of a cotton swab), were also transmissible in series from one rhesus monkey to another and developed more readily on a conjunctiva which had been repeatedly irritated by means of Portland cement powder.

In several monkeys which showed no gross evidence of follicles after inoculation with Noguchi's bacillus, or with tissue removed from monkeys which had developed lesions in response to infection with material taken directly from human trachomatous cases, it was demonstrated that microscopical evidence‡ of changes indicative of a chronic inflammation, or non-apparent infection,[4] were present. These changes consisted of lymphocytic and plasmo-cellular infiltration, edema of the conjunctiva with mucoid degeneration of the cylindrical cell epithelium, and congestion of the capillaries.

By employing the usual subconjunctival method of injection in rhesus monkeys, no evidence of a filtrable virus was found in tissues removed from monkeys whose conjunctivae had developed lesions in response to inoculation with human trachomatous material, or with virulent cultures of *B. granulosis*. No evidence of inclusion bodies was found upon serial passage of human trachomatous material from brain to brain, or from testis to brain of mice or rabbits. This evidence suggests that *B. granulosis,* and not a filterable virus, is concerned in the production of experimental trachoma in rhesus monkeys.

Clinical and experimental data, which will be published in detail,[5] indicate that spontaneous folliculosis of rhesus monkeys is a disease distinct from that which is produced by the inoculation of virulent cultures of *Bact. granulosis.*

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‡ The writer is indebted to Dr. Harvey D. Lamb, Asst. Prof. of Ophthalmic Pathology in this university for these histological reports.

[4] Nicolle, C., *Arch. de l'Inst. Past. de Tunis,* 1925, **14**, 149; and 1928, **17**, 356.

[5] *J. Immunol.,* 1933, in press.

6734

Production of Ventricular Extrasystoles and Pulsus Alternans by Adrenalin.

HOWARD C. PETERS AND LAWRENCE TERRY.
(Introduced by Maurice B. Visscher.)

From the Departments of Physiology and Pharmacology, University of Illinois.

Higgins, Ewing, and McGuigan[1] have produced by intravenous administration of pressor drugs and by clamping the abdominal aorta an apparent slowing of the heart rate in vagotomized dogs under morphine-nembutal anesthesia. As the blood pressure fell from its maximum value, the mercury manometer generally recorded an abrupt change to just one-half the rate at the time.

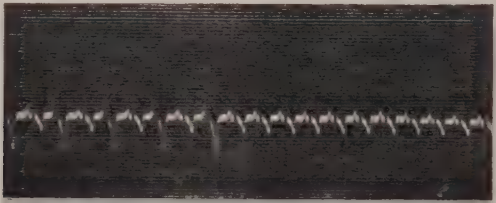

FIG. 1. BIGEMINAL RHYTHM FOLLOWED BY NORMAL BEATS
SPONTANEOUS RECOVERY FROM THE EFFECT OF ADRENALIN.

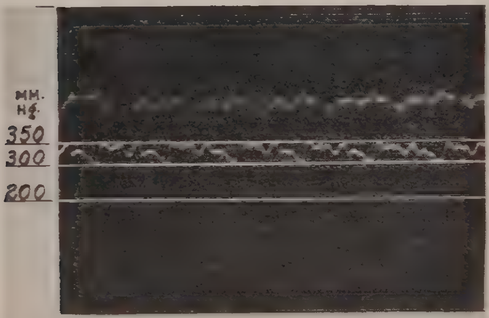

FIG. 2. PULSUS BIGEMINUS
ELECTROCARDIOGRAM AND HÜRTHLE MANOMETER RECORD OF CAROTID PRESSURE DURING HYPERTENSION BY ADRENALIN.

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[1] Higgins, Ewing, and McGuigan, *J. Pharmacol. and Exp. Therap.*, 1932, **44**, 353.

When a certain lower level of pressure was reached, the normal rate was abruptly resumed. We have made electrocardiographic studies of this effect.

In the experiments reported here the typical slowing was produced by the intravenous injection of 5 cc. of 1-10,000 adrenalin into dogs under morphine-nembutal anesthesia. Electrocardiograms were recorded from lead 1. In several experiments the carotid blood pressure was recorded optically along with the electrocardiogram by a Hürthle manometer.

We find that the appearance of a slower rate is due to a pulsus bigeminus (Figs. 1 and 2), consisting of alternate normal beats and beats in which the ventricular contraction is premature. A pulsus alternans is indicated by the carotid blood pressure tracing (Fig. 2) as recorded by the Hürthle manometer.

A bigeminal rhythm due to pressor agents has been observed by Levy[2] in cats under chloroform and under ether anesthesia, although irregularities due to acute hypertension in vagotomized animals have not been thoroughly investigated. The work of Higgins, Ewing, and McGuigan must therefore be regarded as a confirmation and extension of the studies of Levy. It appears from our studies that the bigeminal rhythm may be obtained under nembutal anesthesia as well as under chloroform and ether and that the major condition necessary for its appearance is a sharply elevated blood pressure.

6735

Effect of Rapid Transfusion on Duration of Systolic Ejection of the Left Ventricle.*

EDWARD SIGMAN. (Introduced by Louis N. Katz.)

From the Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, and the Department of Physiology, University of Chicago.

Katz[1] and Wiggers[2] have shown that there is an increase in the duration of the systolic ejection phase following intravenous saline infusion. The increase in duration of this period is independent of any heart rate change and was shown to be due to distention of the heart. However, the possibility remains that this lengthening of systolic ejection might possibly be due to a change in the osmotic pressure of the blood or to a specific action of the sodium chloride. The present research was undertaken to investigate these possibilities.

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[2] Levy, A. G., *Heart*, 1914, **5**, 299.

*Aided by the Frederick K. Babson Fund, Michael Reese Hospital.

[1] Katz, L. N., *J. Lab. and Clin. Med.*, 1921, **6**, 291.

[2] Wiggers, C. J., *Am. J. Phys.*, 1921, **56**, 415, 439.

The technique of Wiggers and Katz[3] for studying the effects of infusions was followed in essentially all details with the following modifications: (1) defibrinated blood was substituted for the isotonic sodium chloride solution, (2) care was taken to keep the temperature of the infused blood the same as that of the animal. Records on bromide paper by optical means were made of the aortic blood pressure and heart volume (Wiggers and Katz[3]).

Summary tables in the 4 successful experiments were made of the systolic, diastolic and pulse pressures in the aorta, and the systolic, diastolic and stroke volumes of the heart, together with the duration of the systolic ejection and the cycle. A typical protocol is shown in Table I. It will be seen that as the transfusion was continued and the heart distended the duration of systolic ejection increased, although the cycle length remained practically unchanged.

TABLE I.
Experiment VI. Three curves measured.

Defibrinated blood added, cc.	Time from onset of exp., min.	Cycle length, sec.	Duration of systolic ejection, sec.	Blood Pressure			Heart Volume	
				Systole mm. Hg.	Diastolic mm. Hg.	Pulse mm. Hg.	Diastolic, cc.	Stroke, cc.
Control	0	0.41	0.14	135	127	8	y	7
100	1	0.40	0.14	154	141	13	y+ 3	13
200	2	0.40	0.14	171	156	15	y+ 8	20
300	3	0.40	0.15	186	164	22	y+10	25
400	4	0.46	0.16	188	161	27	y+10	27
500	5	0.44	0.17	202	171	31	y+18	43
580	9	0.45	0.19	228	172	56	y+20	47

Conclusion. These experiments show that the increase in systolic ejection following transfusion (and in infusion) is due to the distention of the heart.

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[3] Wiggers, C. J., and Katz, L. N., *Am. J. Phys.*, 1922, **58**, 439.

6736

Further Observations on the Effect of Hypophysectomy on Lactation.

J. B. COLLIP, H. SELYE AND D. L. THOMSON.

From the Department of Biochemistry, McGill University, Montreal, Canada.

The removal of the pituitary from a lactating rat leads promptly to a cessation of milk secretion.[1] Immature female rats treated with the anterior-pituitary-like hormone long enough to induce full development of their mammary glands secrete milk when the luteinized ovaries are removed, but not if the pituitary is removed at the same time.[2] These observations seem to support the view of Riddle[3] and others who believe that lactation is controlled by a pituitary hormone, prolactin. Less easily reconciled with this view was the observation[4] that pregnant rats hypophysectomized 10 days before term showed milk secretion for a few hours after parturition. It seemed possible that in these last experiments pituitary function was partly replaced by some stimulus from the pregnant uterus. We found in 10 experiments that if the uterus was emptied by Caesarian section late in pregnancy, lactation set in within 36 hours; similar observations have been made in the guinea pig[5] and the goat.[6] In 6 other experiments, however, in which the pituitary was removed when the uterus was emptied, no milk secretion was observed. This indicates that milk secretion is not possible in the rat if both the fetuses and the pituitary are absent.

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[1] Collip, Selye, and Thomson, *Nature*, 1933, **131**, 56.

[2] Selye, Collip, and Thomson, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 588.

[3] Riddle, Bates, and Dykshorn, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1211.

[4] Selye, Collip, and Thomson, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 589.

[5] Loeb and Hesselberg, *J. Exp. Med.*, 1917, **25**, 305.

[6] Drummond-Robinson and Asdell, *J. Physiol.*, 1926, **61**, 608.

6737

Anterior Lobe or Anterior Lobe-Like Sex Hormone Combinations on Growth of Ovaries of Immature Rats.*

H. L. FEVOLD, F. L. HISAW, A. HELLBAUM AND R. HERTZ.

From the Department of Zoology, University of Wisconsin.

Evans *et al*[1] found that the simultaneous injection of extracts of the anterior pituitary and prolan from pregnancy urine, resulted in a greater effect on the ovaries of immature rats than could be accounted for by the additive effects of the 2 preparations when injected alone. Their theory is that prolan does not act on the ovaries directly but activates the anterior lobe growth hormone so that it becomes the agent which causes the ovarian growth. Leonard[2] found that anterior lobe preparations which contained the gonadotropic substances but were free from growth hormone, would produce augmentation effects when injected in combination with prolan, thus showing that the growth factor was not essential for the reaction. We reported[3] that an anterior lobe luteinizing preparation which produced little or no increase in weight of the immature ovaries when given alone, would augment the action of a given dose of a follicular stimulating preparation. These results, together with others, were interpreted as showing the presence of 2 gonad stimulating hormones. We have now succeeded in separating the 2 principles more completely[4] and have studied the ovarian effects when the 2 are recombined and injected, or used in combination with prolan and the anterior lobe-like hormone of human placentae.

The follicular stimulating extract, in the dosages employed, usually produced only follicular growth, though slight luteinization was present in a few cases. The luteinizing preparation had no noticeable effect on the ovaries of immature rats even when 25 mg. of the luteinizing powder was given. In the present experiments only 5 mg. were used. Castrated horse pituitary powder[5] was also used

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* Assisted by grants from the Committee for Research in Problems of Sex of the National Research Council, the University of Wisconsin Research Funds, and **the Wisconsin Alumni Research Foundation.**

[1] Evans, H. M., Meyer, K., and Simpson, M. E., *Am. J. Phys.*, 1932, **100**, 141.

[2] Leonard, S. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 405.

[3] Fevold, H. L., Hisaw, F. L., and Leonard, S. L., *Am. J. Phys.*, 1931, **97**, 291.

[4] Fevold, H. L., Hisaw, F. L., Hellbaum, A., and Hertz, R., *Am. J. Phys.*, in press.

[5] Hellbaum, A., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 641.

as a source for follicular stimulating hormone. Prolan was prepared from pregnancy urine by acetone precipitation followed by pyridine extraction and the placental hormone from dried human placentae by the same general method used for pituitary powder. Although the preparations from pregnancy urine and placentae have been subjected to the same chemical procedures used in fractionating the anterior pituitary hormones we have obtained no evidence of a separation of 2 principles.

Rats 21 days old were injected for 5 days, twice daily, with equal volume of extract and killed on the sixth day. The ovaries, freed from tubes and bursae, were weighed and the percentage increase over those of normal uninjected controls was calculated.

TABLE I.

		5 mg. Lut. P.		8 R. U. Prolan		8 R. U. Hu. P. H.	
	A	B	C	B	C	B	C
A		none		200		221	
2 R. U. C. H. P.	157	553	252	907	154	1071	183
3/4 R. U. F. S.	50	228	356	560	124		
3 R. U. F. S.	357	1057	196				
4 R. U. F. S.	528	1425	142			1435	91
5 mg. Lut. P.	none			215	none	235	none

Columns A—% increase in ovarian weight for various preparations when given alone.
Columns B—% increase in ovarian weight for various preparations when given in combination.
Columns C—% augmentation over that which can be accounted for by the addition of the increase produced by the preparations when injected alone.
F. S.—Follicular stimulating preparation from sheep pituitary.
C. H. P.—Castrated horse pituitary powder.
Lut. P.—Luteinizing powder prepared from sheep pituitaries.
Hu. P. H.—Anterior lobe-like hormone from human placenta.

The results, presented in Table I, are the averages of at least 6 animals in each case. The luteinizing fraction, which does not increase the ovarian weight when given alone, when added to a given dosage of the follicular stimulating preparation produced a marked augmentation which cannot be accounted for on the basis of an additive effect. When the luteinizing fraction was added to prolan or placental extract, no augmentation occurred. However, when the follicular stimulating preparation was injected with prolan or placental extract marked augmentation resulted. In the experiments with anterior pituitary factors, augmentation is associated with luteinization of the ovaries and is apparently due to the combined action of 2 hormones. Prolan and placental hormone, both strong luteinizers, act like the luteinizing fraction when given in combination with the follicular stimulating fraction. These results do not lend support to the pro-hormone theory as the preparations which were used did not contain growth hormone. Moreover, augmentation does not necessarily depend on the use of prolan as demonstrated with the fractionated sex hormones of the pituitary.

6738

Skin Temperature of the Extremities and Basal Heat Production.

WALTER G. MADDOCK AND FREDERICK A. COLLER.
(Introduced by L. H. Newburgh.)
From the Department of Surgery, University of Michigan.

In the maintenance of a constant body temperature the importance of the skin in the dissipation of body heat is universally recognized. Several studies of normal subjects[1, 2] have shown that in the adjustment to different environmental temperatures there are much greater changes in the skin temperature of the extremities than that of the head and trunk. Therefore, the shift of blood to or from the skin in order to meet the required heat dissipation under the new environmental condition takes place to a much greater extent in the extremities than it does in the head and trunk. Accordingly, we believe that the extremities have a more important part in the regulation of the dissipation of heat from the skin than does the remainder of the body. Such being the case, under a constant environmental temperature differences in heat production ought to be reflected in the skin temperature of the extremities and particularly that of the toes since they show the widest range of normal variations.

This study was carried out under a constant room temperature of 83°F. ± 1°. The basal heat production of 5 normal male subjects wearing only "shorts" was determined by indirect calorimetry, using the Tissot tank and Haldane gas analyzer. The skin tempera-

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[1] Benedict, F. G., Miles, W. R., and Johnson, A., *Proc. Nat. Acad. Sci.*, 1919, **5**, 218.

[2] Coller, F. A., and Maddock, W. G., *Ann. Surg.*, 1932, **96**, 719.

tures of the subjects were recorded during and at the end of the test period with a "Tycos Dermatherm".

TABLE I.

Subject	Data Age	B.M.R.	Basal Heat Production C/M2/HR	Aver. skin temp. great toes
1	28	— 4	37.86	30.8
2	23	— 7	37.03	29.2
3	23	— 9	35.95	28.9
4	25	—11	35.02	27.8
5	24	—16	33.17	25.9

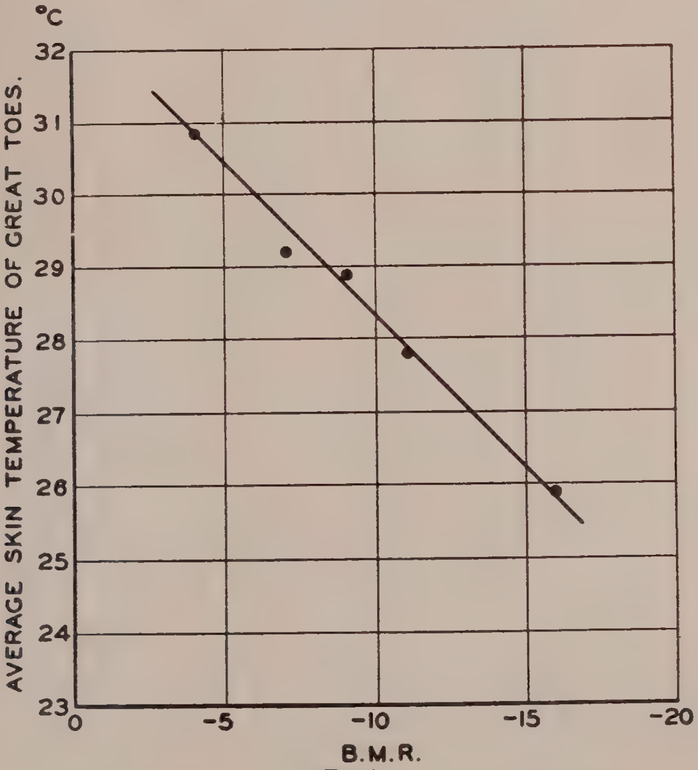

FIG. 1.

Relationship of skin temperature of great toes to basal metabolic rate.

Conclusion. Under the constant environmental temperature for the male subjects studied, a simple linear relationship was found between the basal heat production and the average skin temperature of the great toes.

6739

Excessive Anterior-Pituitary-Like Hormone and Variations in Oestrin in the Toxemias of Late Pregnancy.

GEORGE VAN S. SMITH AND O. WATKINS SMITH.
(Introduced by Harvey Cushing.)

*From the Fearing Research Laboratory, Free Hospital for Women, Brookline, Mass.**

Quantitative determinations of the anterior-pituitary-like gonad-stimulating hormone and of oestrin have been made on the sera and urines of 27 women, both normal and toxemic, during late pregnancy, *i. e.*, from 7½ months to term. The Ascheim-Zondek and Allen-Doisy methods have been employed. Ten cases were normal, 14 were diagnosed toxemia without convulsions and 3 had eclampsia.

The following procedure was carried out in every instance. A mixed 24-hour specimen of urine was collected and measured. Forty cc. of blood were allowed to clot and the serum separated. For the Ascheim-Zondek test the amounts of urine and sera to be assayed were precipitated with 5 volumes of 95% alcohol, allowed to stand overnight and centrifuged. The precipitate was washed with ether and taken up in saline for injection into female rats 3 weeks old. A test was considered positive only if corpora lutea were grossly visible. In establishing the hormone content of each urine and serum 5 to 20 rats were used, depending upon how well the results agreed. For the Allen-Doisy assay extracts of the serum were made by precipitating the proteins with 95% alcohol, extracting the precipitate repeatedly with alcohol and ether, evaporating the combined supernatant fluids and taking up the residue in saline for injection into mature spayed female rats. The urine was not extracted. Six to 24 rats were used for establishing the oestrin content of each specimen, the smaller number being often necessary in the assays on sera because of the limited amount of material. The rats used were frequently tested with oestrin of known potency. A full oestrous smear in half the rats into whom a given amount of specimen was injected was considered positive.

In both Ascheim-Zondek and Allen-Doisy determinations the amount of hormone in the 24-hour volume of urine was calculated on the basis of the smallest quantity that would give a positive test. The use of 24-hour volumes of urine deserves especial emphasis because of the obvious fallacy of attempting to compare results of any quantitative urine determinations without some gauge of the concentration of the specimens analyzed.

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* The Mrs. William Lowell Putnam investigation of the toxemias of pregnancy.

In the Ascheim-Zondek tests on 10 normally pregnant women, all but 2 of whom were 3 weeks or less from term, as much as 1.5 cc. to 2.5 cc. of serum was required to give a positive result, the average figure being 2.05 cc. The urinary excretion varied from 325 to 900 r.u. in 24 hours, the average figure being 540 r.u.

In the Ascheim-Zondek tests on 14 patients diagnosed as toxemic without convulsions, all but 3 of whom were 3 weeks or less from term, the smallest amounts of sera that would give positive results lay between 0.07 cc. and 1.0 cc., the average figure being 0.51 cc. The urinary excretion varied from 1230 to 13000 r.u. in 24 hours, the average figure being 4216 r.u.

It was impossible to collect 24-hour urines on the 3 eclamptic patients. Two of them were positive for the gonadotropic hormone with as little as 0.2 cc. of serum and the third was positive with 0.025 cc.

The 10 normally pregnant women showed excretion of oestrin varying between 4200 and 7500 r.u. in 24 hours, the average being 6000 r.u. In every instance 3 cc. of the sera of these women gave full oestrous smears, and in all but 2 of them 2 cc. stimulated full oestrous.

There were strikingly wide variations in the urinary excretion of oestrin by the 14 patients diagnosed as toxemic without convulsions, the figures lying between 310 and 10,000 r.u. in 24 hours, with an average of 3800 r.u. The excretion of only 4 of these patients lay within normal limits. Nine of the remaining 10 excreted less than the normal amount. Of their sera, 9 were completely negative and 5 were positive for oestrin in 3 cc. amounts. Three of these 5 were completely negative in 2 cc. amounts.

Of the 3 eclamptic sera, one gave a positive Allen-Doisy test with 2 cc., one gave a positive test with 3 cc., but negative with 2 cc., and the third gave a negative with 3 cc.

It would appear, therefore, that the blood and urine of toxemic patients in late pregnancy contain excessive amounts of the anterior-pituitary-like hormone, and wide variations in oestrin with a decided tendency toward low levels. Preliminary experiments with hypophysectomized rats indicate that the excessive gonadotropic hormone of these toxemias may be hypophyseal in origin.

6740

Influence of Acid and Phosphate on Metastatic Calcification.

DORAN J. STEPHENS AND DAVID P. BARR.

From the Department of Medicine, Washington University, and the Barnes Hospital, St. Louis.

Metastatic calcification in otherwise apparently healthy soft tissue has been occasionally observed accompanying destructive bone lesions in cases in which there is associated renal disease. The factors responsible for this deposit have not been entirely evaluated. Rabl[1] fed mice alternately acid and alkaline diets rich in calcium and phosphorus and observed typical metastatic calcification in kidneys, heart, stomach, lungs and arteries. He explained the deposition of calcium on the basis of assumed rapid alterations in the alkalinity of the blood and resulting variations in calcium solubility. Butler,[2] Dreyfuss,[3] and Kleinmann[4] confirmed Rabl's observations but found that alternation was not necessary and that acid diets rich in calcium and phosphorus, when taken alone, were also followed by metastatic calcification. In their experiments it is not clear to what extent the deposit is due to the acidity as such and how much must be attributed to the fact that acidity was produced by phosphoric acid. The following observations were undertaken to determine whether high phosphate intake was necessary to the production of metastatic calcification according to the method used by Rabl.

The diets were modeled after those of Rabl. Each diet contained 100 parts of sugar and 100 parts of dried whole milk, containing 1.357% of calcium and 1.660% of phosphorus. Further adjustments were made as follows: (1) High phosphorus, high calcium acid diet—10 parts of calcium phosphate and 10 parts of 85% phosphoric acid were added. (2) High phosphorus, high calcium alkaline diet—10 parts of calcium phosphate, 200 parts of saturated solution of sodium acetate and 300 parts of cooked mashed potato were added. (3) High phosphorus, high calcium neutral diet—10 parts of calcium phosphate were added. (4) High calcium acid diet—10 parts of calcium lactate and 10 parts of ammonium chloride were added. (5) High calcium alkaline diet—10 parts of calcium lactate, 200 parts of saturated solution of sodium acetate, 300 parts of cooked mashed potato were added. (6) High phosphorus acid diet—5 parts of disodium phosphate and 10 parts of 85% of phosphoric acid were added.

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[1] Rabl, C. R. H., *Klin. Wochenschr.*, 1923, **2**, 202.

[2] Butler, M., *Proc. N. Y. Path. Soc.*, 1924, **24**, 79.

[3] Dreyfuss, W., *Beit. z. path. Anat. u. allg. Path.*, 1927, **76**, 254.

[4] Kleinmann, H., *Biochem. Z.*, 1928, **196**, 161.

Young adult white rats were fed one or another or a combination of these diets for a period of 15 days, after which they were killed. The following organs were studied microscopically: lung, liver, kidney, heart, pyloric portion of stomach, spleen, pancreas, thoracic aorta, skeletal muscle, skin, and in a few instances testicle, lymph node, brain and adrenal. Sections were fixed in 4% neutral formalin; paraffin sections were stained with silver nitrate and hematoxylin and eosin, and with silver nitrate and alum carmine.

Forty young adult white rats were used and all survived until the end of the experimental period. The results of feeding the various diets and combinations of diets are shown in Table I. Soft tissue

TABLE I.
Soft Tissue Calcification in Rats.

Diet	No. Animal	Kidney	Heart	Stomach	Artery and Location	Other Organs
High Calcium	1	+++	0	0	0	0
High Phosphorus	2	++++	+	++	++ Pulmonary	0
Acid	3	++	+	+	++ Renal	0
4 Rats	4	++	0	0	+ Pulmonary	0
					+ Stomach	0
					0	0
High Calcium	1	+	0	0	+ Pulmonary	0
High Phosphorus	2	+++	0	0	+ Pulmonary	0
Alternating acid	3	+	0	0	++ Pulmonary	0
and alkaline diets	4	+	0	0	0	0
every 2 days						
4 Rats						
High Calcium High Phosphorus Alkaline—5 Rats	all	0	0	0	0	0
High Calcium High Phosphorus Neutral—5 Rats	all	0	0	0	0	0
High Calcium Acid—12 Rats	all	0*	0	0	0	0
High Calcium Alternating acid and alkaline diets every 2 days—6 Rats	all	0	0	0	0	0
High Phosphorus Acid—6 Rats	all	0	0	0	0	0
Normal Rats	all	0	0	0	0	0

* In one kidney section there were 3 tiny questionable calcium concretions in tubules. Duration of each experiment—15 days.

calcification was observed only with the high calcium, high phosphorus acid and the high calcium, high phosphorus alternating diets, and was more marked and widespread with the former. No deposit was found when phosphate was not present in the diet. Of the individual organs, the kidney and pulmonary artery were most frequently involved; the most extensive calcification was seen in the kidneys.

The calcium was deposited as gross concretions, easily seen under low power of the microscope as dense black masses in the stained sections; the calcium deposits were all apparently extracellular. In all organs, the tissue surrounding the calcium concretions was the site of a cellular reaction consisting chiefly of infiltration of lymphocytes. In the heart muscle, in addition to the lymphocytic reaction, there was necrosis and early scarring in the immediate vicinity of the calcium deposit. In the kidneys, the most extensive deposition of calcium was noted in the region of the junction of medulla and cortex and extended into both of the latter; calcium was deposited for the most part in tubules; to a lesser extent in the interstitial tissue. No deposits were seen in the glomeruli. In the stomach sections, calcification was noted in the muscular coat and in one case in the wall of the small artery. In the pulmonary, renal and gastric arteries which showed calcification, the deposits were located beneath the intact intima, in the muscular layer. No deposits were seen in any of the sections of the aorta. Several of the lung sections showed calcium deposits in cartilages of the larger bronchi; this was also seen in several of the control rats and, therefore, was not considered significant.

The calcium deposits could be removed from sections by treatment with 5% hydrochloric acid before staining with silver nitrate. In unstained sections containing large amounts of calcium, no gross or microscopic bubbling could be detected upon treatment with 5% hydrochloric acid. This was interpreted as indicating that significant amounts of carbonate were not present and that the calcium was deposited chiefly as the phosphate salt. Iron stains were done on a number of sections; in all organs showing calcium with the silver nitrate stains, small amounts of iron were demonstrated in the areas of calcium deposition.

As controls, the organs of 6 healthy young adult white rats were sectioned and stained; in none of them was any calcification observed except in bronchial cartilages, as noted above.

Serum calcium and serum phosphorus values were determined on blood samples obtained at the time the rats were sacrificed. There was no gross deviation from the normal in the animals in which soft tissue calcification was observed.

The probable importance of phosphate in the deposit of calcium in soft tissues has been shown by Smith and Elvove,[5] who found in their experiments with viosterol poisoning that the relationship of calcification in tissue to phosphate values in serum could be clearly established. High serum phosphate accompanied by only a slight increase in serum calcium generally resulted in great calcification of soft tissues. On the other hand no abnormal deposition of calcium was found in any case in which the serum phosphate values were low. Shelling[6] found that animals fed with a minimum of calcium and a high phosphorus ration were extremely susceptible to viosterol and exhibited most profound calcification of soft tissues. He showed further that increased phosphorus retention characterized the metastatic calcification observed with hypocalcemia in parathyroidectomized dogs. Phosphate retention is a prominent feature in the terminal stages of nephritis. Following overdosage with parathormone or with irradiated ergosterol there is in the terminal stages an increase in serum phosphate. Since calcium metastases are recognizable only at autopsy, it seems possible that in these conditions the deposit occurs late and only after phosphate retention has become an important factor.

The experiments here reported indicate that in the development of metastatic calcification an excess of calcium, of phosphorus and of acid is necessary, and that if any one of these is not present in excess soft tissue calcification does not occur.

It is suggested that conditions of these short-term experiments in rats may be fulfilled for the development of metastatic calcification in certain patients who have a rarefying bone disease and a renal lesion, the former acting as a source of excess calcium, the latter resulting in acidosis and phosphate retention.

Summary. 1. Rats fed high calcium, high phosphorus, acid or alternating acid and alkaline diets for a period of 15 days, show extensive soft tissue calcification, confirming previous experiments by others. 2. With acid or alternating diets high in calcium but not rich in phosphorus, no calcification takes place. 3. With an acid diet rich in phosphorus but without added calcium, calcification does not occur. 4. Neutral or alkaline diets rich in calcium and phosphorus do not result in calcium deposition. 5. An excess of calcium phosphorus and acid is necessary for the production of metastatic calcification under the conditions of these experiments. Neither acidity nor rapid alternation between acidity and alkalinity is sufficient to cause significant calcification unless both calcium and phosphorus are present in excess. 6. The presence of iron was demonstrated wherever calcium deposits occurred.

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[5] Smith, M. I., and Elvove, E., *Pub. Health Rep.*, 1929, **44**, 1245.

[6] Shelling, D. H., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 298.

6741

Current Intensity Factor in Electrocution of Paramaecia.

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From the Biology Department, St. Louis University.

The purpose of the present work is to determine in *absolute values* the intensity of current required to pass through a Paramaecium to disrupt it.

The organism, *Paramaecium caudatum*, cultured for 5 to 6 days in hay infusion, which consisted of Timothy hay boiled for 5 minutes in 100 cc. of distilled water and diluted to 500 cc. was introduced into a glass tube of known diameter and length. The tube was placed horizontally on a disc of hard rubber, the ends being immersed in big drops of culture medium into which the electrodes carrying the current (D.C.) were also introduced. The disc, placed on the stage of the microscope, was hollowed in the middle so as to allow the light from the condensor to pass through. Rheostats and milli- or microammeters, in series, completed the circuit. When a paramaecium came in sight in the tube the circuit was closed and the data taken. Selecting only those experiments in which the organisms were disrupted instantaneously (in less than 1/5 of a second) a "Band of killing intensities", extending from the intensities that killed about 5% to those that killed about 95%, was established for a set of tubes varying widely in diameter.

The following results were obtained: (cross sectional area of the tubes less cross-sectional area of the organism, in mm^2, in line A; band of killing intensities in microamperes, in line B).

A	0.14	.047	.048	.057	.277	.410	.488	.579	.827	1.126
B	7-10	9-13	9-11	12-19	35-68	44-62	59-66	85-95	100-166	144-186

A	1.192	2.258	3.786	4.128	5.461	6.502
B	130-136	353-375	539-631	650-677	854-887	1042-1097

The average of the 2 extreme intensities of the "band", plotted on the curve. (Fig. 1) show an evident linear relation.

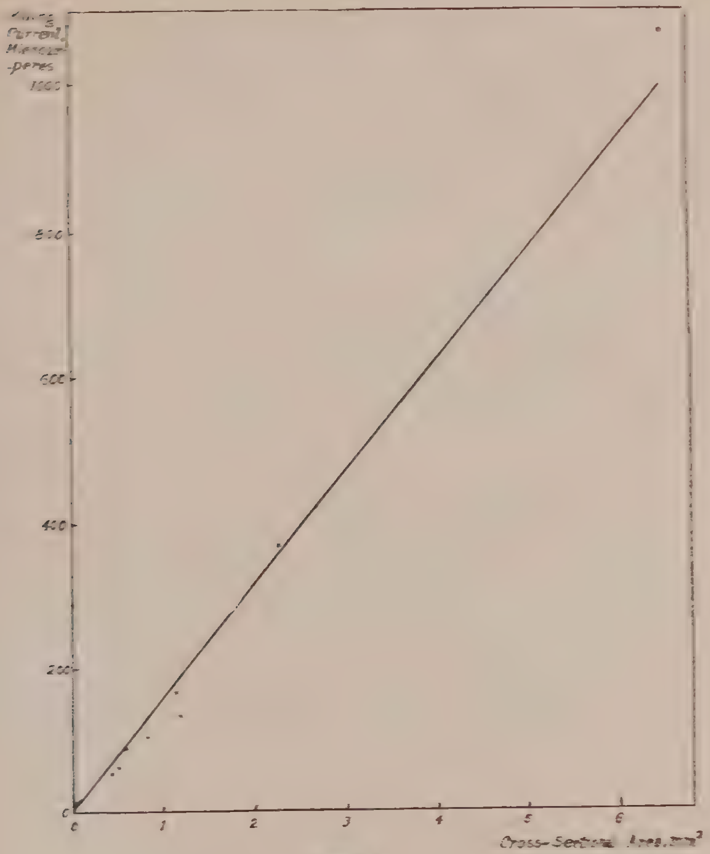

FIG. 1.

The system consisting of a paramaecium in suspension in its medium, in a thin glass tube through which a current is made to pass, can seemingly be compared to a system of 2 conductors in parallel, one being the organism and the other the column of liquid having the length of the paramaecium and surrounding it. Let then a, r, k, and i represent, respectively, the mean cross-sectional area, the total resistance, the resistivity of the paramaecium and the intensity of the current passing through it; a', r', k', and i' the corresponding quantities for the column of liquid above defined; and I the total intensity of the current passing through the tube. The formulas of conductors in parallel: $I = i + i'$ (1) and $ir = i'r'$ (2) give

$I = i + \frac{ik}{} a'$ (3), which means that the relation between the cross-sectional area of the tube less the cross-sectional area of the organism, and the intensity of the current passing through the tube—a part of which will kill the paramaecium—must be a linear one.

Analysis of the results leads then to the following conclusions:

1. The experimental curve is in good agreement with the above assumption on the distribution of the current through the organism and its surrounding medium, although it does not exclude some other interpretation.

2. If a' is made equal to zero the formula (3) reads $I = i$, which means that the intersection of the ordinate and the curve gives the intensity of the current passing through the paramaecium and killing it. That intensity, which may extend from 1 to 10 microamperes in the above curve, was found to be near 1 microampere in some later experiments.

3. If i is known and if r' can be determined, the formula (2) will give the resistance r of the paramaecium. The resistance r' of the medium was measured with the Leeds and Northrup conductivity measuring equipment. The resistance of the paramaecium was then found to be about 700,000 ohms and its resistivity 600 ohms. (The resistivity of the hay tea was 1896 ohms, the length of the organism has been estimated at 0.23 mm. and its mean cross-sectional area at 0.002 mm^2.)

I mention the present procedure as a means of measuring the resistance of organisms of the type paramaecium.

6742

Toxicity Study of Potassium Perrhenate.

L. C. HURD, J. K. COLEHOUR AND P. P. COHEN.
(Introduced by H. C. Bradley.)

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Rhenium, the third homologue of the manganese family, has been known to a few scientists for relatively few years. Although discovered in 1925, it was not obtainable until 1930. The relationship of rhenium to manganese and its chemical similarity to osmium made it appear worthwhile to investigate the physiological effects of the element. The investigation was necessarily limited to a few direct experiments since the material was expensive and available in but small quantities. The rhenium salt used was potassium perrhenate.

In the analysis of the tissues for presence of the element, a quartz prism spectrograph was employed. The rhenium lines were compared with a standard and a rough approximation was possible as to the presence of a trace or a considerable amount.

I. *Toxicity studies.* A group of 9 mice weighing 15-30 gm. were given intraperitoneal injections of potassium perrhenate in doses corresponding to 0.05-3 mg. of rhenium. All the animals but one recovered within 12 hours. The one fatality occurred immediately following injection and was in all probability due to an accident in injection. The immediate effect of injection was that of water intoxication, since the relative insolubility of the salt demanded the injection of relatively large amounts of solution. This effect was experimentally controlled.

A group of 6 rats were then given intraperitoneal injections in doses equivalent to 2.5-50 mg. of rhenium. No unusual effects were observed in the week following. At the end of the week the rats were each given an injection containing 5 mg. of rhenium, followed by 9 daily injections containing 2.5 mg. of rhenium per injection. One rat weighing 245 gm. received in one injection an equivalent of 50 mg. of rhenium, corresponding to 200 mg. per kilo body weight. The minimum lethal dose of manganese calculated as metal is given as 5-6 mg. per kilo body weight for the rabbit.[1] In the several weeks following no unusual effects were observed in any of the rats.

II. *Fate of potassium perrhenate.* In an attempt to determine the fate of the salt, 2 rabbits were given intravenous injections of the salt in physiological saline. Rabbit No. 1 received 2 injections containing 25 and 45 mg. of rhenium in a half hour interval. Rabbit No. 2 received 2 injections containing 100 mg. of rhenium each at one half hour intervals. They were killed about an hour and a half after the first injection and the following organs removed, desiccated, and examined spectrographically for the presence of rhenium.

	No. 1	No. 2		No. 1	No. 2
Brain	—	—	Kidney	+	+
Testes	+	++	Liver	+	+
Urine	+++	+++	Spleen	+	+
Heart	+	+	Adrenals	+	—

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[1] Hanzlik and Sollman, Introduction to Experimental Pharm., 1928.

III. *Physiological effects.* In an attempt to measure any physiological effects, a 20 kg. dog was anesthetized with ether and the blood pressure and heart rate measured. An intestinal plethysmograph was also employed. A solution containing 250 mg. of rhenium was injected into the femoral vein. The changes observed indicated a very slight splanchnic dilatation. However, this effect was not subject to adequate experimental control to warrant interpreting this effect as being due to the injected salt.

Continuation of the toxicity study is being held up pending the development of a satisfactory quantitative method for the determination of rhenium in the presence of organic matter. The work will be resumed at an early date.

6743

Effect of Hypophysectomy on Growth of the Walker Rat Tumor.

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Since hypophysectomy is known to lead to cessation of body growth, it seemed interesting to investigate its effect upon the growth of a tumor such as the Walker rat tumor. Twelve out of 25 young albino rats bearing tumor transplants 8 to 12 days old were therefore hypophysectomized (by Doctor Hans Selye), the remainder being kept as controls. The area of the largest cross-section of the tumors was measured by sketching them upon squared paper with the aid of calliper measurements; the total weight of the rats was also followed. The results are summarized in Table I.

TABLE I.

Days after operation	Hypophysectomized (Average of 12 rats) Tumor area, cm.^{2}	Hypophysectomized (Average of 12 rats) Total wt.	Control (Average of 13 rats) Tumor area, cm.^{2}	Control (Average of 13 rats) Total wt.
0	3.8	176	3.3	188
12	8.2	170	10.9	203
18	10.7	171	15.4	216
24	13.1		19.3	

Hypophysectomy evidently retards but does not prevent the growth of the tumors; but the total weight of the rats and their tumors remained stationary (as in hypophysectomized rats without tumors) or slightly declined. With one exception, in which the growth-curve suggests that the operation was incomplete, the hypophysectomized rats died in from 21 to 36 days, and in none of them did the tumor attain 50 gm. weight. In the unoperated rats the rate of increase of the size of the tumors was obviously greater, and the total weights increased. These rats were killed off at intervals as the experimental animals died; not one of them died, although in many cases the tumor exceeded 100 gm. in weight. It was noted that in the hypophysectomized rats, in the last few days of life, the total weight decreased; in more than half of the cases the size of the tumor also decreased before death.

The volume and weight of the tumors may be roughly estimated from the measured cross-sections, allowing for slighter development in the third dimension; this method has been checked in another series in which the tumors were weighed *post mortem*. It appears to be sufficiently reliable, on the average to permit the conclusion that in the present series the weight of the rats *minus* their tumors decreased, after the first few days. Thus in the hypophysectomized rats the gain in weight of the tumor is almost exactly equal to the loss of weight of the rest of the body, whereas in the normal rats the growth of the tumor is absolutely and relatively greater, the loss of weight of the rest of the body being approximately the same as in the hypophysectomized rats. One possible interpretation may be that in the normal rat the growth of the tumor is supported both by the existing healthy tissues and by the assimilated food-stuffs, whereas in the hypophysectomized rat it takes place solely at the expense of the tissues. It was found histologically that the tumors borne by hypophysectomized rats showed larger areas of necrosis than the control tumors; no other difference was observed. Further experiments on the effect of various hypophyseal extracts on the growth of this tumor are in progress. The present findings are in agreement with those on a mammary carcinoma reported by Ball, Samuels and Simpson.[1]

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[1] Ball, H. A., Samuels, L. T., and Simpson, W., *Am. J. Cancer*, 1932, **16**, 351.

6744

Spectrographic Analysis of the Nessler-Ammonia Reaction.

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Various attempts have recently been made to develop a direct method for estimating ammonia by correlating the ammonia content of a solution with the intensity of the orange-red color produced in it by the action of Nessler's reagent.[1] Such a method would be expected to yield the most satisfactory results when monochromatic light at or near the wave-length of maximum absorption is employed, but so far as we are aware the absorption spectrum of the Nessler-ammonia color has not hitherto been investigated. We have, therefore, measured this property both for Nessler's solution itself (pre-

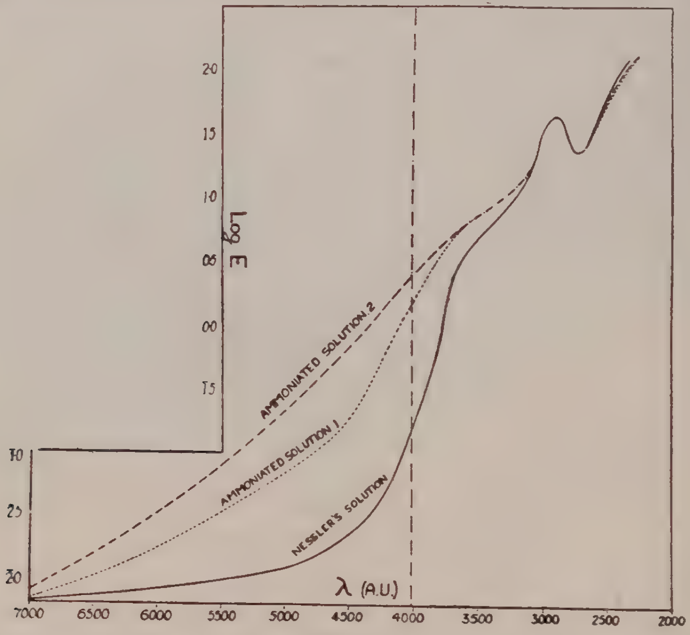

FIG. 1.

Solution 1 contained 0.08 mg. NH_3 in 100 cc.; solution 2, 0.2 mg. in 100 cc. E = 100 d/cl, where d = optical density of the solution; c = number of cc. of Nessler's solution per litre; l = thickness of liquid.

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[1] *Cf.* Urbach, *Biochem. Z.*, 1932, **252**, 292, 406.

pared according to the directions of Folin), and for 2 colored solutions containing different proportions of ammonia, the solution in each case being stabilized by the addition of gum ghatti.

The results are shown in Fig. 1. The curve for Nessler's solution is similar to that recorded for yellow-colored K_2HgI_4,[2] and consists of a strong band at 2900 A.U., 1000 times more intense than the absorption in the visible spectrum, with a "step-out" at 3500 A.U. The addition of ammonia produces no change in the position or intensity of the head of this absorption band, and does not seriously affect the absorption at long wave-lengths, but that in the blue and near ultra-violet regions appears to be enhanced. The orange-red color of the ammoniated solutions cannot, therefore, be attributed to any change in the maximum of absorption, and we suggest that it is more probably the result of light-scattering than of light-absorption.

The following evidence can be cited in support of this view: 1. The colored solutions are colloidal and flocculate rapidly unless a stabilizer is added. 2. The substitution of ammonia for potassium in the unionized double-salt would not be expected to produce such a change in the "shape" of the absorption band without affecting its position and intensity. 3. Serious deviations from Beer's law were observed in this region of the spectrum. 4. The yellow color of colloidal sulphur solutions results from scattering, and not from absorption.[3] 5. An examination of the colored solutions under the ultra-microscope indicated the presence of large particles with strong scattering. 6. The intensity of the light scattered by a particle is proportional to the square of its volume while the absorption depends only on the first power, so that as particle size increases, the scattering becomes relatively more important than the absorption. A comparison can thus be drawn between the intensification of the Nessler color during flocculation of unstabilized solutions and that of As_2S_3 during precipitation, as studied by Boutaric and Vuillaume.[4]

It is therefore concluded that until more is known of the optical properties of ammoniated Nessler's solutions, great caution must be exercised in attempts to estimate the ammonia by methods involving the use of interpolation curves which are based on measurements of the intensity of the light transmitted through them.

Our thanks are due to Professor T. M. Lowry, F.R.S., for his kind interest and for permission to carry out these measurements in his laboratory.

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[2] Goldstein, Thesis, Cambridge University, 1926.

[3] Keen and Porter, *Proc. Roy. Soc.*, A, 1914, **89**, 370.

[4] Boutaric and Vuillaume, *J. Chim. Physique*, 1925, **21**, 247.

6745

Reticulocytes of Normal Albino Rats.*

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The literature on nutritional anemia reveals numerous discrepancies in the observations of different investigators. Some workers report that iron alone exerts no hematopoietic effect while others, including ourselves,[1] have obtained partial remission of anemia with such supplements. Although the milk anemic rats used by Drabkin and Miller[2] failed to regenerate when iron only was administered, prompt recovery from anemia ensued if glutamic acid supplemented the iron. Elvehjem, Steenbock and Hart[3] were unable to confirm this observation. Our studies[1] have shown that glutamic acid was ineffective in the case of anemic rats reared from normal mothers, although it exerted a positive effect on hemoglobin regeneration in the case of anemic rats bred from mothers which had been fed a protein-low diet (cystine-deficient). The iron and glutamic acid preparations used in our studies were apparently free from copper and the dry klim, which served as the basal ration, showed no greater copper content than the milk employed by the Wisconsin workers. Furthermore, the same batch of klim and the same preparations of iron and glutamic acid were employed in our 2 series of experiments. Therefore, the dissimilarity in the response of our 2 groups of rats cannot be ascribed to variations in the ration or supplements but rather to differences in the anemic animals or their progenitors.

It is quite possible that some of the discrepancies in the results on milk anemia reported from various laboratories may be due to differences in the copper reserves of the animals. Nevertheless, it was thought desirable to look for other criteria which could demonstrate differences in stock rats used for breeding anemic animals.

The percentage of reticulocytes in the blood of the stock rats employed by Dr. D. L. Drabkin† for the production of his anemic animals was quite different from that obtained by us on some of the rats from our stock colony. Accordingly, systematic reticulocyte determinations on our normal stock animals were made and these data correlated with age, growth, blood hemoglobin level and the red blood cell count.

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* In cooperation with the Research Department of the Calco Chemical Co., Bound Brook, N. J.

[1] Stucky, C. J., *J. Biol. Chem.*, 1932, **97**, xiii.

[2] Drabkin, D. L., and Miller, H. K., *J. Biol. Chem.*, 1931, **90**, 531; 1931, **93**, 39.

[3] Elvehjem, C. A., Steenbock, H., and Hart, E. B., *J. Biol. Chem.*, 1931, **93**, 197.

† Personal communication and discussion of data presented in a demonstration at the meeting of the Federation of American Societies for Experimental Biology, Philadelphia, April, 1932. These data will be published shortly.

Our colony was of the Wistar strain and had been inbred for several generations. The animals were fed a stock ration which was found to be adequate for the nutritional requirements of the rat. The composition of the diet was as follows: Rolled oats 30, whole milk powder 15, dried yeast‡ 10, wheat embryo 10, meat scraps (Swift's 65%) 20, casein 10, cod liver oil 3, sodium chloride 0.5, sodium bicarbonate 0.75 and calcium carbonate 0.75%. In addition, fresh green lettuce was fed at frequent intervals.

Estimations of the hemoglobin, red blood cells and reticulocytes were made at approximately weekly intervals on groups of rats from birth until the animals were 3 months old. Blood was obtained by clipping the tails. Hemoglobin was determined colorimetrically using a calibrated Newcomer disc. "Total hemoglobin" was calculated from blood volume[4] (total Hb = blood volume × Hb/100

TABLE I.
Changes occurring with age in the reticulocytes and other blood constituents.

Age	No. rats	No. litters	Sex		Wt.	Blood Estimations				
			♂	♀		Hb	Tot. Hb	R. B. C.	Reticulocytes	
days					gm.	gm. per 100 cc.	gm.	millions per c.mm.	%	millions per c.mm.
0	3	1	2	1	6	10.3	.05	2.7	61	1.7
7	3	1	1	2	16	8.6	.10	3.2	42	1.3
14	3	1	2	1	31	7.0	.15	4.4	31	1.4
21	12	4	5	7	47	8.0	.26	4.8	25	1.2
21	22	4	14	8	43	—	—	—	24	—
21	31	7	11	20	47	7.5	.24	—	—	—
28	12	4	5	7	68	10.7	.47	5.7	18	1.0
28	12	3	3	9	81	11.2	.58	—	—	—
28	4	1	1	3	75	—	—	—	20	—
37	15	6	6	9	100	12.0	.76	7.0	11	.8
43	9	4	5	4	119	13.6*	.99*	7.1*	6	.4
49	12	5	4	8	148	15.0	1.34	8.5	4	.3
56	12	5	4	8	152	15.0	1.37	9.8	3	.3
67†	12	5	5	7	196	15.9‡	1.83‡	9.1‡	3	.3
82†	8	4	2	6	213	17.0	2.09	9.3	2	.2
96†	7	3	4	3	277	17.1	2.67	9.7§	2	.2

* Average of 5 rats. † Mean age. ‡ Average of 10 rats. § Average of 6 rats.

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‡ Obtained through the courtesy of Mr. Leo Wallerstein of the Wallerstein Laboratories, New York, N. Y.

[4] Donaldson, H. H., The Rat, Philadelphia, 1924, 287.

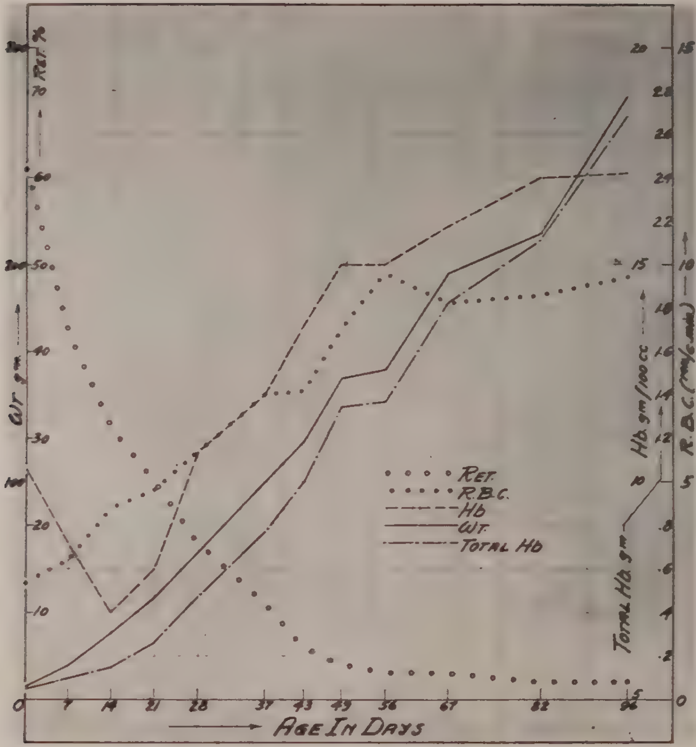

FIG. 1.

gm.). For the preparation of smears for the reticulocyte counts, a slight modification of the usual technique was introduced. In the customary method, the blood is mixed directly with the dried stain on the slide. In our procedure one drop of a 0.2% solution of sodium citrate is dried on the slide in a spot free from the brilliant cresyl blue. Then a drop of blood is mixed first with the citrate and then with the dye. The remainder of the procedure is carried out as usual. In this way uniform blood smears may be obtained easily.§

The data are presented in the table and chart. There is a rapid decrease in the number of reticulocytes from birth (approx. 60%) until the animals are 2 months old. Thereafter their number remains fairly constant (2 to 3%). It is interesting to note that the well-known drop in the hemoglobin level during the nursing period is associated with a progressive decrease in the percentage of reticulocytes and an increase in the number of red blood cells. Only a few rats were used for the reticulocyte estimations during the first 3 weeks of life but it is believed that the figures are fairly representative. When the rat pups are weaned at 21 days of age, the reticulocytes have dropped to a 25% level. The values for the other blood constituents are in accord with those in the literature, the maximum hemoglobin being 17 gm. % (approx.) and the maximum red blood cell count being 9-10 millions for our young stock adults.

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§ The complete details will be described elsewhere by Miss E. M. Kapp.

Since young adult rats, 2 to 3 months old, show a very low and constant reticulocyte count (2 to 3%), it would appear that such a criterion might be used as another index of the physiological condition of the stock colony. Thus, values above this level might indicate a condition of potential anemia. Hence, it might serve as an additional basis of comparison for the stock rats of different laboratories. It is conceivable that such information might help in the interpretation of some of the conflicting data on nutritional anemia reported by different investigators.

Summary. The percentage of reticulocytes in the blood of young rats bred from an original Wistar strain varying in age from birth to 3 months was determined. These data have been correlated with growth, hemoglobin level, total hemoglobin and red blood cell count. The drop in the hemoglobin level during the nursing period is associated with a progressive decrease in the number of reticulocytes. Rats from our colony 2 to 3 months old show 2 to 3% (.2 to .3 millions per c.mm.) of reticulocytes. The significance of the number of reticulocytes in young adult stock animals as a possible criterion of potential anemia has been indicated.

6746

Picrotoxin-Stramonium Antagonism.

LILLIAN SHUTTER AND HAROLD B. MYERS.

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The actions of picrotoxin and stramonium on the upper central nervous system suggest the possibility of their possessing some degree of mutual antagonism. The site of the principal action of picrotoxin upon the central nervous system has been localized by serial sections of the brain in various species of experimental animals. As described in a standard text[1]: "The seat of action thus seems to move upward as the higher parts of the central nervous system become more developed, the chief effects arising from the spinal cord and medulla and optic lobes in the frog and from the cerebrum and midbrain in mammals." Picrotoxin effectively antagonizes the depressant action of barbituric acid derivatives as shown by Maloney and Tatum.[2, 3] Regarding the chief location of action of the barbituric acid derivatives, Bastedo states[4] "The action of these drugs is most pronounced on the midbrain."

Stramonium has come to be the chief drug used in relieving the symptoms of Parkinsonism, following its introduction by Juster.[5] Clinical reports[6, 7] indicate an action by stramonium in this condition that is superior to the action of its known alkaloidal constituents, individually. McAlpine[8] has described pathologic changes in the *substantia nigra* as the chief lesion in post-encephalitic Parkinsonism. The lesions described are considered responsible for the symptoms of motor imbalance relieved by the action of stramonium.

Available information would therefore suggest the possibility of an antagonism in the chief central nervous effects of picrotoxin and stramonium, particularly in the region of the midbrain. Interest in such an antagonism is heightened by the increasing use of stramonium and an increasing interest in the action of picrotoxin. It was decided to test the effect of stramonium upon the convulsant action of picrotoxin.

The experiments were carried out upon the white rat. Picrotoxin was injected subcutaneously in 0.5% strength in distilled water by means of a syringe calibrated in 1/100 cc. graduations. The minimal convulsive dose of picrotoxin was found to be 0.4 mg. per 100 gm. weight. All rats injected with this dose developed convulsions. Five of 8 rats injected with 0.35 mg. picrotoxin per 100 gm. weight developed convulsions. None of the rats injected with 0.3 mg./100

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[1] Cushny, A. H., Pharmacology and Therapeutics. Lea and Febiger, Philadelphia, 1928.

[2] Maloney, A. H., Fitch, R. H., and Tatum, A. L., *J. Pharm. and Exp. Therap.*, 1931, **41**, 465.

[3] Maloney, A. H., and Tatum, A. L., *J. Pharm. and Exp. Therap.*, 1932, **44**, 337.

[4] Bastedo, W. A., Materia Medica, Pharmacology and Therapeutics. W. B. Saunders and Co., Philadelphia, 1932.

[5] Juster, M. E., *Revue Neurologique*, February, 1925.

[6] Menard, O. J., and Hurxthal, L. M., *New Eng. J. Med.*, 1931, **205**, 759.

[7] Gragman, L., *Med. J. and Rec.*, 1930, **131**, 21.

[8] McAlpine, D., *Brain*, 1926, **49**, 525.

TABLE I.
Minimal Convulsive Dose of Picrotoxin.

Rat No.	Wt.	Picrotoxin mg./100 gm.	Result	Time min.
97	43	0.25	No convulsion	
19	80	0.30	” ”	
93	50	0.30	” ”	
96	48	0.30	” ”	
98	47	0.30	” ”	
99	50	0.30	” ”	
100	49	0.30	” ”	
101	69	0.35	Convulsion	27
102	54	0.35	”	30
20	144	0.35	No convulsion	
25	55	0.35	” ”	
26	60	0.35	” ”	
92	48	0.35	Convulsion	28
95	42	0.35	”	23
106	160	0.35	”	25
1	90	0.40	”	20
7	57	0.40	”	19
8	61	0.40	”	20
9	53	0.40	”	21
21	157	0.40	”	25
46	36	0.40	”	39
47	36	0.40	”	27

In each instance the first dose of Picrotoxin received by the animal.

gm. developed convulsions. These results are presented in Table I.

All convulsive reactions occurred between 19 and 30 minutes following the injection of picrotoxin (with the single exception of rat No. 46, delayed until 39 minutes). Rats appearing on the verge of convulsions began to show signs of recovery about 40 minutes after receiving picrotoxin, appearing fully recovered in 120 minutes or less. Though one might anticipate from these facts an early and complete freedom from the effects of picrotoxin, there is an evident increased susceptibility to the action of the drug, rapidly acquired, though doses are spaced 24 hours or even several days apart (Tables II and III). Results in Table I are therefore restricted to reactions following the first injection of picrotoxin.

Rats weighing 225 to 325 gm. develop convulsions with relatively small doses of picrotoxin. Though the number of animals was not

TABLE II.
Increased Sensitivity to Picrotoxin Injected Daily. Rat No. 93.

Date	Wt.	Picrotoxin mg./100 gm.	Result	Time min.
10/11	50	0.30	No convulsion	
10/12	50	0.30	Convulsion	37
10/13	51	0.30	”	14
10/14	52	0.25	”	28
10/15	52	0.20	No convulsion	

TABLE III.

Increased Sensitivity to Picrotoxin Injected at Intervals.* Rat No. 7.

Date	Wt.	Picrotoxin mg./100 gm.	Result	Time min.
10/27	57	0.40	Convulsion	19
11/13	100	0.40	”	16
11/17	110	0.40	”	?
11/25	110	0.40	”	24
12/ 3	132	0.35	”	20
12/28	163	0.30	”	20

* Second-Fifth injections given with stramonium in protective amounts. I was interested in seeing if the convulsive seizure itself had anything to do with the increased sensitivity toward picrotoxin developed as a result of repeated doses of that drug. I protected several rats against the convulsions by means of a prior injection of a protective amount of stramonium. It did not seem to make any difference in the sensitivity of the animal. The second dose on Nov. 13th, being the 6th dose without stramonium, the remaining doses in natural sequence.

sufficient to justify setting a definite figure, it was evident that approximately 0.25 mg. picrotoxin per 100 gm. weight is a convulsive dose for rats of this weight group. Large rats require a longer time (30 to 40 minutes) to develop convulsions, than do rats of less than 100 gm. weight.

Rats of 20 gm. weight or less respond with convulsions with a dose of picrotoxin of 0.4 mg./100 gm., the minimal convulsive dose indicated in Table I, and become convulsive in less than 20 minutes.

The alcohol content of a U.S.P. tincture of stramonium was removed by evaporation in a water bath until the volume was 40% of the original. Distilled water was added to restore the original volume. This solution of stramonium injected subcutaneously, failed to cause any symptoms, at least so far as activity was concerned, until a dose of 0.5 cc./100 gm. weight was reached, when voluntary movements seemed slightly decreased. But little increase in this effect was noticed as the dose was increased until a dose of 1.0 cc./100 gm. was reached. The animal would then exhibit spasmodic, exaggerated movements while otherwise appearing depressed. The exhibition of mixed spasmodic movements and depression was increased when the dose of the stramonium solution was increased to 2.0 cc./100 gm.

The alcohol-free tincture of stramonium acted as an antagonist of limited power when injected at least 10 minutes prior to the picrotoxin. The limitation of capacity in antagonistic action on the part of stramonium was apparently due to an increased irritability of the central nervous system caused by doses of the stramonium solution of 1.0 cc./100 gm. weight and larger. Table IV indicates the antagonistic action of stramonium toward the minimal convulsive dose of picrotoxin.

TABLE IV.
Antagonism of Stramonium (alcohol-free tincture) Toward the Minimal Convulsive Dose of Picrotoxin.

No. Rats	Stramonium*	Picrotoxin.	Result
10	.4 cc./100 gm.	.4 mg./100 gm.	Convulsions in 9 animals
10	.5 cc./100 gm.	.4 mg./100 gm.	Convulsions in 1 animal
10	.6 cc./100 gm.	.4 mg./100 gm.	No convulsions

* Each dose of stramonium given 10 minutes prior to picrotoxin injection.
Doses of 1.0 to 2.0 cc./100 gm. weight of the stramonium solution did not prevent the development of convulsions in rats given 0.5 mg. picrotoxin/100 gm.

Conclusions. 1. Stramonium exhibits an antagonistic action toward the convulsant stimulation of picrotoxin in rats. 2. The antagonistic action of stramonium is limited by an evident stimulant effect in large doses upon the central nervous system. 3. It is suggested that the stimulant action of stramonium in large doses upon the central nervous system may be, in part at least, an explanation of the therapeutic value of stramonium upon the partially destroyed cells of the *substantia nigra*, in the large doses found desirable in Parkinsonism. 4. There is a possibility that the picrotoxin-stramonium antagonism might serve as a means of the biological standardization of stramonium.

6747

A Piezo Electric Myograph.

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In the experiments here reported, the piezo-electric properties of Rochelle Salt crystal have been employed to record the tension developed by contracting muscle. For references to the literature on the piezo-electric effect the reader may consult the comprehensive bibliography of W. G. Cady.[1] The widespread use of the piezo-electric effect for measuring forces and pressures lead to its consideration as a means of recording muscular tension. It offers an instantaneous response without friction, inertia or moving parts, which is particularly well adapted to the measurement of the tension devel-

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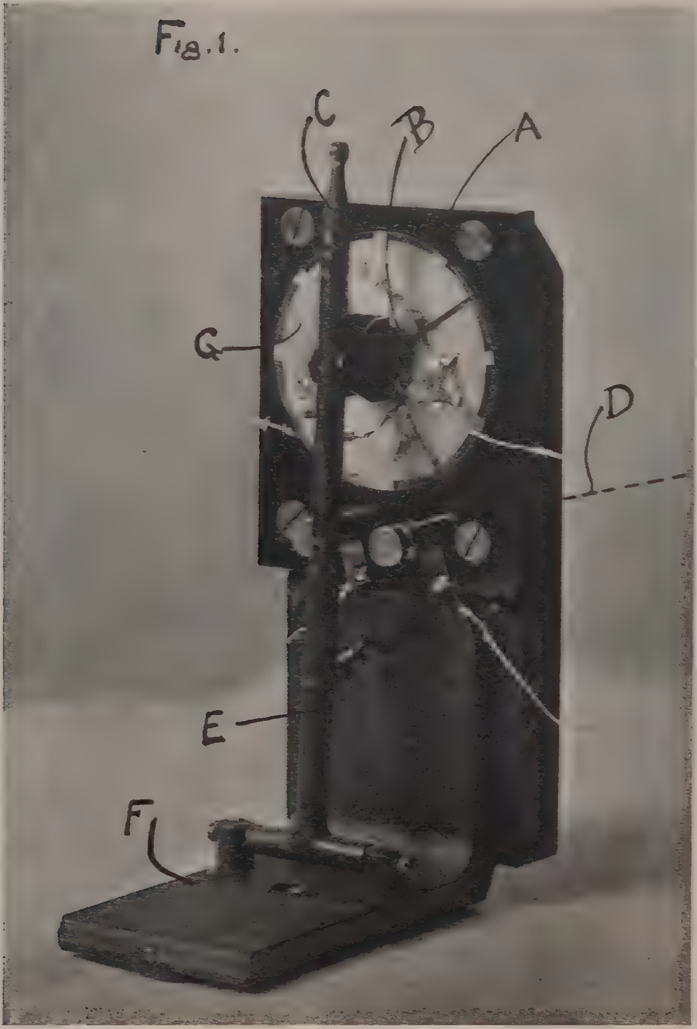
[1] Cady, W. G., *Proc. Inst. Radio Engineers*, 1928, **16**, 521.

Fig. 1.



oped in striated muscle in an isometric contraction in which tension rises to a maximum in about 30 sigma. Although our work is incomplete, the results obtained indicate the useful possibilities of the method.

Through the courtesy of C. Baldwin Sawyer of the Brush Development Laboratories, Cleveland, we were supplied with a Rochelle salt crystal designed especially for muscle physiology. This crystal, G, Fig. 1, is a flat cylinder 6.4 cm. x 0.6 cm., made from 2 identical plates each about 0.3 cm. thick, cemented together. These plates, which were derived from a specially grown crystal, were cut in such a way that the flat surface of the plate is parallel to the b and c axes of the crystal and perpendicular to the a axis. Before being cemented together the b and c axes of the 2 plates are rotated 90° from one another. There are quadrant electrodes of tin foil on each face and between the plates. The crystal was mounted in a frame of

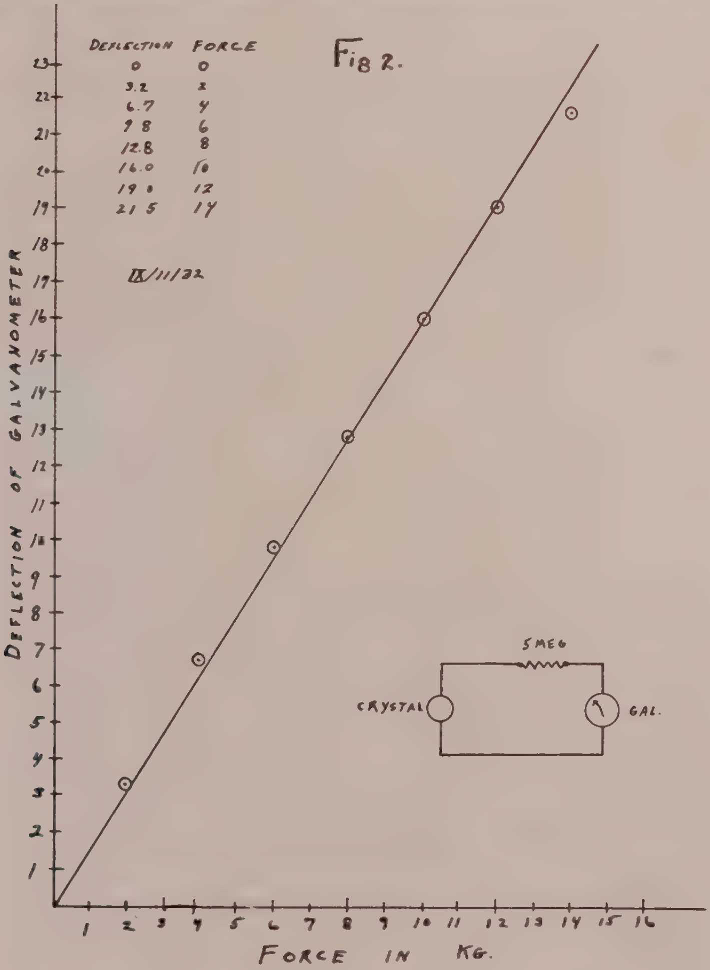
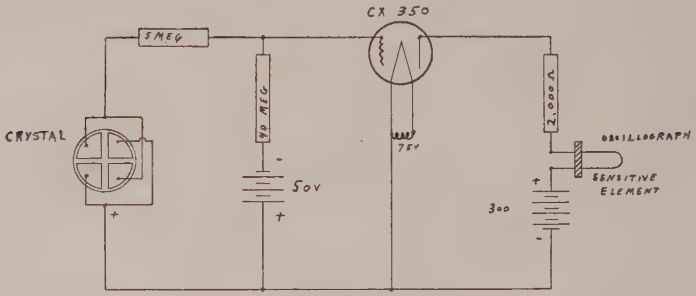


Fig 2.

DEFLECTION	FORCE
0	0
3.2	2
6.7	4
9.8	6
12.8	8
16.0	10
19.0	12
21.5	14

IX/11/32

Fig. 3.



Bakelite, A (Fig. 1), and held in place by the pressure of a Bakelite disc, B, 1 cm. in diameter which was attached to the rod C, upon which force was exerted through the wire, D, which is attached to the muscle. A small spring, E, exerted a slight but sufficient pressure to hold the crystal snugly in place. The Bakelite frame is attached to a heavy angle-iron base, F, which may be clamped onto a slotted piece of channel iron.

The calibration of the crystal with respect to impressed force and E.M.F. output is shown in Fig. 2. This calibration was made by means of a Leeds and Northrup ballistic galvanometer Type P with 5 megohms in series. For measuring the E.M.F. output a second method was used in which the crystal was connected (Fig. 3) with the grid of a type 350 radio tube. Variations in the plate current with the grid potential were recorded by means of a Westinghouse oscillograph. The potentials developed by the crystal frequently reached 180 volts. This suggested the use of the inverted vacuum

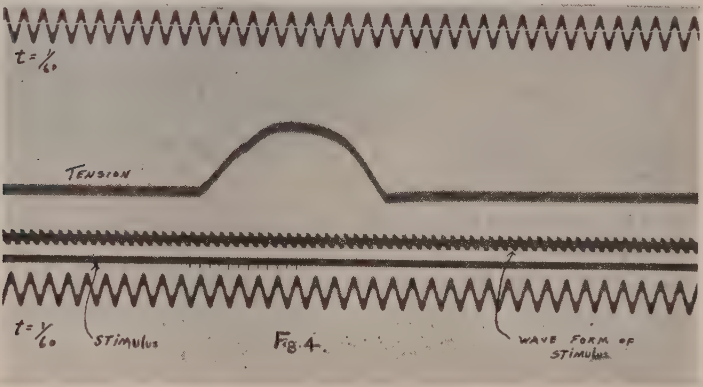


Fig. 4.

tube circuit with type 245 tube which operated quite satisfactorily. Since this work was done a new General Electric tube, type FP 110, has come on the market. The characteristics of this tube make it probable that, used in connection with the oscillograph, much more exact data may be obtained.

A piezo-electric, oscillographic, myogram is shown in Fig. 4. This was obtained by the hookup shown in Fig. 3 from the gastrocnemius muscle of a decerebrate cat (No. 492, ♀, wt. 1.8 kg.). The muscle was prepared with the tuber calcanei attached to the wire, D of Fig. 1. The initial tension was estimated at 200 gm. The muscle was stimulated through the tibial nerve by a series of 12 stimuli derived from a rectified 110 volt, 60 cycle a. c. current giving 120 pulses per second. The top and bottom lines of the record are time in 1/60 of a second. The second line is the oscillographic record of the changes in tension in the muscle registered by the E. M. F. which is developed in the crystal by the force exerted on it by the contracting muscle. The oscillographic record of the wave form of the stimulus is shown in the third line. Although the wave form of the stimulus is shown continuously on the record, only the 12 pulses shown in the fourth line were allowed to reach the nerve and cause muscular contraction. The absence of a "nose", plateau and angle in the myogram is in agreement with the findings of Cooper and Eccles[2] in which they show that a myogram obtained with a frictionless torsion wire myograph is devoid of discontinuities. The rather slow ascent of the myogram may be influenced by leakage from the crystal. As regards the permanence of the crystal, Sawyer[3] states "the crystal is not hygroscopic. * * * * The life of the crystal is indefinite, and it will not fail from atmospheric variations, or from 'fatigue'."

Since writing this article we have found two references in the literature. A piezo-electric myograph in which a quartz crystal has been employed, has been described by A. M. Monnier (*C. R. Acad. Sci.*, 1931, **195**, 1487). The instrument is figured but no myogram is shown. J. Sosnoski (*Am. J. Physiol.*, 1929, **90**, 524) reported at the 13th International Physiological Congress, experiments with a quartz crystal the emf. of which actuated a string galvanometer. No records are shown.

Also tube F.P. 110 has been tried and found to work very satisfactorily.

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[2] Cooper, S., and Eccles, J. C., *J. Physiol. Proc. Soc.*, 1930, **1**.

[3] Sawyer, C. B., *Proc. Inst. Rad. Eng.*, 1931, **19**, 2029.

6748

Biological Action of Strongly Positive Redox Systems.

ELLA H. FISHBERG.

From the Chemical Laboratory, Beth Israel Hospital, New York.

Oxidation-reduction systems which are extremely positive from a biological point of view are continually circulating in the animal organism, but the poising effects of the redox systems of middle range such as hemoglobin are so efficient that the effects of these positive systems formed as intermediate products in the metabolism of phenyl alanine and tyrosine components of protein pass unnoticed. Only in pathological states, such as alkaptonuria, with its accompanying ochronosis, in cases of poisoning with overdosage of acetanilid, aniline, benzene, phenol, etc., can they be appreciated.

We have measured the oxidation-reduction potential of homogentisic-benzoquinone acetic acid as representative of these systems and as a substance known to circulate in the blood in alkaptonuria. This was done with bright platinum electrodes in strongly buffered solutions at fixed temperature of 25° with exclusion of air above pH 3. Table I is typical of the results. Quinone-hydroquinone was measured in the same way. Graph 1 shows the relation of pH and E_h of these systems, and for purposes of orientation hemoglobin-methemoglobin as measured by Conant[2] is included in the graph.

It has been shown by the work of Küster[1] and Conant[2] that the

TABLE I.

Oxidation of 20 mg. homogentisic acid in buffer (NaCl + HCl) with bichromate at same pH. End Point 18 cc. pH 1.984.

Oxidant cc.	Oxidant %	.06 log S_r/S_o	E_h Observed	E'_o Calculated	Deviation
2	5.5	.370	.5328	.5698	.0002
3	11.1	.272	.5429	.5701	.0001
4	16.7	.209	.5493	.5702	.0001
6	22.2	.163	.5544	.5700	.0000
7	27.8	.124	.5584	.5708	.0008
8	44.4	.029	.5667	.5696	.0004
9	49.9	.000	.5700	.5700	.0000
10	55.5	.029	.5733	.5703	.0003
11	61.0	.058	.5755	.5696	.0004
12	66.6	.089	.5787	.5698	.0002
14	77.7	.168	.5862	.5699	.0001
16	88.8	.270	.5971	.5701	.0001
17	94.4	.368	.6070	.5702	.0002
			Aver.	.5700	

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[1] Küster, W. J., *Z. Physiol. Chem.*, 1910, **66**, 244.

[2] Conant, J. B., and Fieser, L. F., *J. Biol. Chem.*, 1924, **56**, 595.

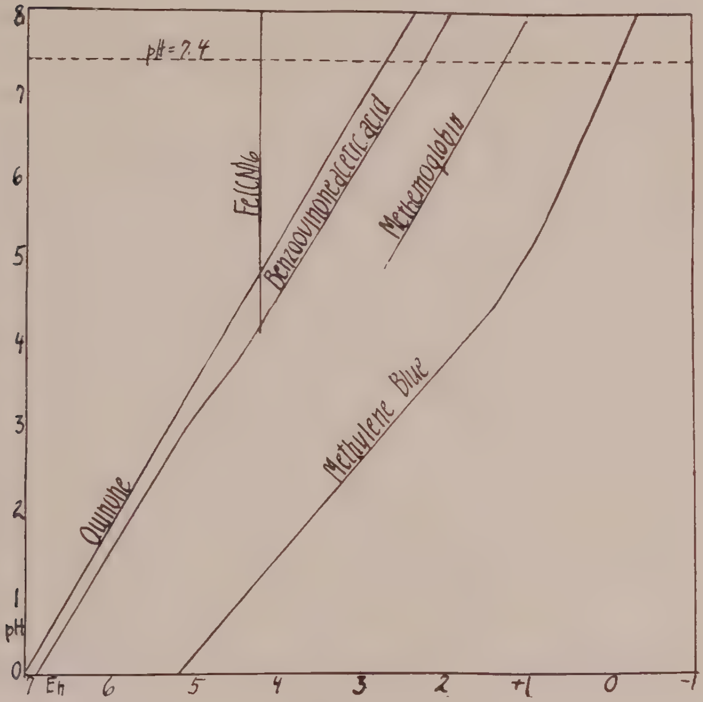

FIG. 1.

iron in methemoglobin is in the ferric state contrasted to the ferrous iron present in hemoglobin and oxyhemoglobin. The potential of the hemoglobin system is 0.15 V at body pH. Graph 1 shows that the potential of the strongly positive systems are all above 0.25 Volts at body pH. In poisoning by aromatic substances circulation of polyhydroxylated phenols causes a shift in the hemoglobin-methemoglobin equilibrium in favor of methemoglobin. This is the characteristic symptom of poisoning by these agents. The injection of acetanilid, resorcinol, etc., into dogs results in the formation of as much as 50% methemoglobin. In the clinical entity known as "enterogenous cyanosis" we have violent intestinal upsets followed by intense cyanosis in which the blood can be shown to contain methemoglobin in large quantities. This is probably due to the action of the phenol-quinone systems liberated from the damaged intestine.

Hence to the manifold functions of hemoglobin in the organism must be added its poising effect on higher oxidation systems. It must of course be taken into account that though these systems are present in very small quantities the molecular weight of hemoglobin is 68,000 while the quinones are around 100, so that theoretically a concentration of only 20 mg. per 100 cc. of blood would be sufficient to turn the entire hemoglobin of the body into methemoglobin. The poising effect of the hemoglobin is sufficient for the normal physiological amounts formed in protein metabolism, but added amounts in aniline poisoning can and do cause the appearance of methemoglobin.

It is significant in this connection that the black pigment deposited in ochronosis is found entirely in the cartilages and sclera. These are the places where the poising effect of the hemoglobin must be absent. Hence the oxidized phase may form and this polymerizes instantly at body pH to form the melanin-like aggregates characteristic of the disease.

6749

Functional Heart Mechanisms.

C. C. GUTHRIE.

From the Department of Physiology and Pharmacology, Medical School, University of Pittsburgh.

Studies on frogs, turtles, rabbits, cats, dogs, and man over more than 20 years and participated in by numerous associates, including Doctors Kruse, Waddel, Koenig, Criep, Hover, McLain, and Lauler have led to numerous conclusions:

I. Contraction normally begins in the superior vena cava and spreads to auricles and ventricles. The evidence and proof include: *a*. Direct observation. *b*. Simultaneous recording of contraction of veins, auricles, and ventricles alone and combined with galvanometric recording of electrical variation. (Fig. 1.) *c*. Sequence of recovery from vagus inhibition. (Fig. 2.) *d*. Transmission of premature venous contractions to auricles and ventricles. (Fig. 2.) *e*. Photographic (cinema) recording.

In man, the evidence is mainly derived from polygraphic and galvanometric records, the hitherto occasionally observed, but unexplained U-wave corresponding to the venous electrical variation observed in lower animals. It may be observed in most subjects by increasing galvanometer sensitivity as by slacking the fibre, and

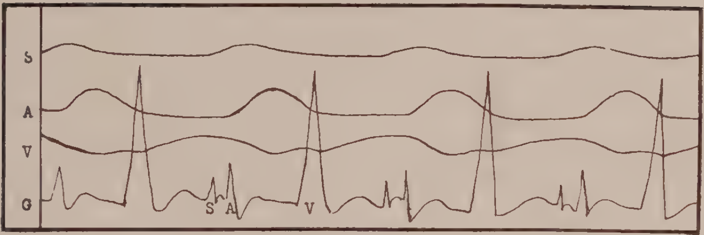

FIG. 1.
Simultaneous mechanical and galvanometer record of terrapin sinus, auricle, and ventricle.

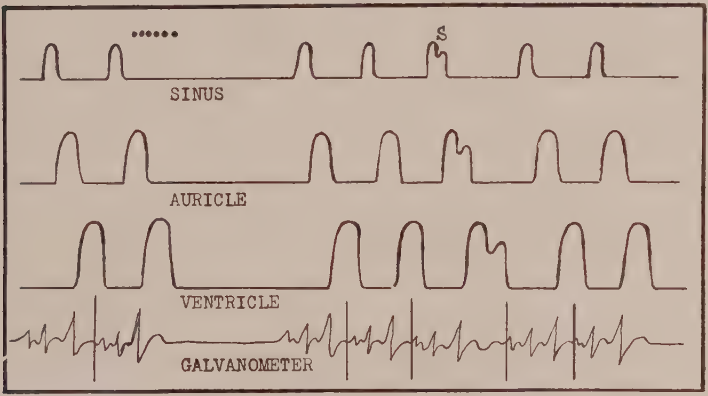

FIG. 2.
Diagram of evidence of origination of excitation in sinus of mammals.
..... = stimuli to vagus nerve. S = stimulus to sinus.

placing one electrode in the supra-sternal notch, right supra-clavicular fossa or in the mouth. Standard figures showing time relations of heart activities recorded simultaneously by different means as by polygraph and galvanometer are confirmatory.

II. Heart abnormalities and experimental irregularities as arhythmias, alternation, etc., are usually due to changes in fundamental properties of tissues or their time relations. Other cause, as failure of conduction or spread of excitation—"block" in the current sense—at most, is rare. Due regard to the composite character of gross manifestations, *i. e.*, the algebraic summation of unit or segmental response, is of utmost value in heart studies as in all other tissue studies.

III. Differences in different species studied are mainly, if not wholly, quantitative.

IV. Venous and auricular galvanometer records, when taken as entities, show form and characteristics very similar to ventricular ones, provided the galvanometer is adjusted for adequate sensitiveness.

V. Simple valves in veins and arteries adequately regulate flow, owing to the physical characteristics of those structures. Auricles and ventricles not possessing like characteristics require more complicated ones, *i. e.*, to withstand pressures to which they are subjected, the free edges of their valves are attached to the papillary muscles whose contractions seemingly are timed and regulated through the Purkinje or "bundle" tissues in hearts possessing such tissue.

These conclusions are based upon: (1) Direct observation. (2) Indirect observation: *a.* Mechanical registration of contraction. *b.* Galvanometric registration of electrical manifestations. *c.* Simultaneous coordinated observation and registration of contractile and electrical manifestations. (3) Controlled and coordinated observation and measurement of fundamental properties of venous, auricular, and ventricular tissues, as irritability, latent, contraction, relaxation, and refractory periods, normal and under changed conditions as temperature, drugs, etc.; and after mutilation as cutting, clamping, etc. (4) Cinema film,

Since the function of the heart is to circulate the blood and since this is wholly dependent upon contraction (Fig. 3), this has been

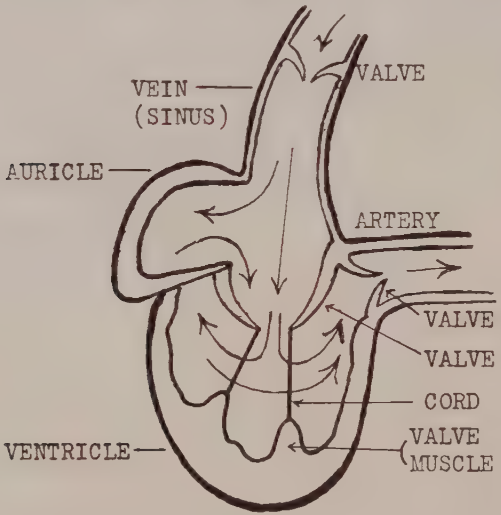

FIG. 3.

Diagram of functional, *i. e.*, blood-pumping unit of heart. The veins contract while the ventricles are relaxing, rushing blood into the auricles, which in turn contracting, discharge their contents into the ventricles, which in turn contract and expel their contents into the arteries, valves establishing one way flow.

the basic index for conclusions—electrical manifestations, etc., being evaluated and judged as concomitant and secondary phenomena and of significant practical value only insofar as their meaning is revealed by contractile phenomena.

These conclusions are more in line with the older observations and conclusions than with current beliefs and teachings.

6750

Observations on Behavior of the Anthocyan Pigment from Concord Grapes in the Animal Body.

M. K. HORWITT. (Introduced by L. B. Mendel.)

From the Laboratory of Physiological Chemistry, Yale University.

The anthocyans are not uncommon constituents of our diet. They are especially abundant in berries, grapes and grape products. Nevertheless, facts concerning the metabolism of these pigments are meager. In the following experiments we investigated both the alimentary and parenteral fate of the anthocyan from Concord grapes.

The anthocyan necessary for the experiments was prepared as the chloride according to the method described by Anderson.[1]

(A) 50 mg. of the pigment in saline solution, subcutaneously injected into large rats (450 gm.), resulted in the excretion of the dye in the urine. The specimen at the end of the first day was intensely colored, almost black; the next day's urine had a slightly bluish tinge. Proof of the presence of the dye was obtained by adding to the urines a drop of HCl, which changed the color to a deep red. Other tests confirmed the presence of the pigment. Apparently, a considerable portion of the pigment was excreted without undergoing any change.

(B) In order to determine whether or not the compound was absorbed from the intestine, quantities of 100 mg. of the anthocyan chloride were fed to starved rats by stomach tube. The pigment was not excreted in the urine over a period of 4 days, although the addition of 0.2% of pigment directly to the urine gave the mixture a decided pink color. Heating the urine with acid did not increase the pigmentation. At the same time, qualitative examination of the feces showed some pigment to be present.

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[1] Anderson, R. J., *J. Biol. Chem.*, 1924, **61**, 97.

(C) The possibility that the pigment was partially destroyed by intestinal bacteria was investigated. Small amounts of different bacterial media, to which a trace of anthocyan chloride had been added, were inoculated with saline extractions of human feces and incubated. No change in color was apparent after periods of one, 2 or 3 days. Suitable controls, which differed only in that they contained saline extract of feces that had been sterilized, were run.

(D) A dog with a simple permanent gastric fistula of the valve type[2] was anesthetized with amytal. The common bile duct, pancreatic duct and the thoracic duct were cannulated. A milk suspension of 500 mg. of dye was placed in the duodenum by means of a catheter into the gastric fistula and through the pyloric sphincter. "Secretin" was injected intravenously in order to stimulate the flow of pancreatic fluid. Examination of the bile, pancreatic fluid, urine and lymph during the following 2 hours showed that the pigment was not present. Under the conditions of this experiment, there was little, if any, absorption of the pigment through the intestinal wall. Intravenous injection of the anthocyan chloride resulted in its appearance in the bile and urine in 20 minutes and in the lymph in 50 minutes. No pigment was found in the pancreatic fluid, the intraocular fluid, or in the exudations from a cisternal puncture.

(E) The benzene rings in the anthocyan chloride have been suspected as a source of hippuric acid in the normal metabolism of this pigment. Even though absorption of the compound in appreciable quantities was questionable, experiments were planned to determine whether or not there was any measurable increase of conjugated benzoic acid compounds in the urine after ingestion of the pigment. Milk suspensions of 500 mg. of the pigment were administered to rabbits by stomach tube, but the total benzoic acid in the urine, as determined by the method of Kingsbury and Swanson,[3] showed no significant deviation from the normal. The normal excretion of total benzoic acid on a milk diet, determined for each rabbit, averaged about 60 mg. per day. If the fate of ingested anthocyans is the same in the human species as in rabbits, then these pigments probably do not augment the hippuric acid excretion in man.

It is noteworthy that the urine and feces of the rabbits which were fed the anthocyan chloride became highly pigmented. Colorimetric

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[2] Technique for preparing a gastric fistula described by A. J. Carlson: *Control of Hunger in Health and Disease,* Univ. of Chicago Press, 1916, 42.

[3] Kingsbury, F. B., and Swanson, W. W., *J. Biol. Chem.,* 1921, **48**, 13.

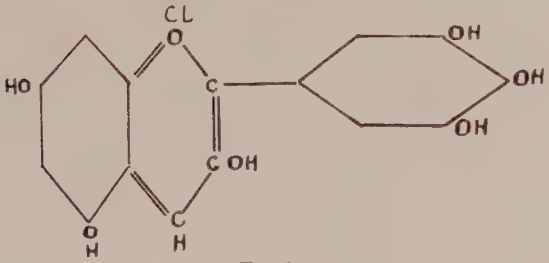

FIG. 1.

Delphinidin chloride. Illustrates nucleus in grape anthocyans.

examination of these urines showed that from 1 to 2% of the dye was excreted through the kidney. The doses used, however, were greatly in excess of the amounts ordinarily ingested.

Conclusion. The above experiments indicate that the anthocyans are not easily absorbed from the intestine. The small quantity which does pass through to the circulation appears to be excreted by the kidney in its unchanged form.

The author wishes to express his gratitude to Dr. R. J. Anderson, who suggested this problem, and Drs. R. W. Jackson and G. R. Cowgill for their valuable advice and assistance.

6751

Effect of Ferric Chloride Injections on Spread of Tuberculosis from Site of Subcutaneous Inoculation.*

VALY MENKIN.

From the Department of Pathology, Harvard University Medical School.

Previous studies have demonstrated that repeated intravenous injections of a 0.25% solution of ferric chloride crystals in tuberculous rabbits are followed by an accumulation of iron in the caseous areas of the lungs.[1, 2] Subsequent studies revealed that concomitant with this accumulation of iron in tuberculous areas there was a definite increase in the survival time of infected rabbits.[3, 4] When control and experimental animals were sacrificed at given intervals of time after inoculation with tubercle bacilli, in the majority of instances the extent of the lesion in experimental animals was distinctly less than that found in the controls.[4] The conclusion drawn was that with the accumulation of iron in tuberculous areas, the course of development of the disease is protracted in the rabbit.

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* Aided by a grant from the Delamar Mobile Research Fund.

[1] Menkin, V., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1020.

[2] Menkin, V., and Menkin, M. F., *J. Exp. Med.*, 1931, **53**, 919.

[3] Menkin, V., *J. Exp. Med.*, 1932, **55**, 101.

[4] Menkin, V., *Am. J. Med. Sci.*, 1933, **185**, 40.

Further experiments were set up to determine whether the repeated administration intravenously of ferric chloride solution would retard the dissemination of tubercle bacilli and hence the progress of the disease when microorganisms were inoculated into a subcutaneous area. Prior to the present study inoculation with tubercle bacilli had been performed only by the intravenous route.

A saline suspension of a bovine culture of tubercle bacilli was introduced into the subcutaneous tissue of the abdomen of each of 12 rabbits. Repeated intravenous injections of ferric chloride in concentration of 0.25% were started in 6 of the rabbits, immediately following the inoculation with tubercle bacilli. The remaining 6 tuberculous animals were kept as controls. The method of injecting ferric chloride and the precautions used throughout the course of administration to avoid lethal effects, have been described.[3, 4] Throughout the period of injections, death from embolism was no doubt prevented in the majority of instances by the fact that, as previously pointed out, the precipitate formed by the interaction of 0.25% ferric chloride with blood serum redissolved.[5] As a rule death resulting from intravenous administration failed to occur when the amount of 0.25% ferric chloride injected at one time was below 4 or 5 cc. The injections were always given very slowly. The ferric chloride solution was kept on ice when not in use. The solution was heated slightly prior to each injection.

After variable intervals of time ranging from 35 to 94 days, following inoculation with tubercle bacilli, a control and an experimental rabbit having had the disease for the same length of time would be sacrificed. A careful post mortem examination was performed. The spread of the disease from the site of inoculation in each rabbit was studied by determining, in the gross, the extent of tuberculosis in the peritoneal lining, the omentum, small intestine, colon, kidneys, spleen, liver, diaphragm and lungs. In the majority of control animals, tuberculosis was observed in practically all of these organs. In the experimental animals, on the contrary, tuberculosis was less widely distributed, being in some cases entirely absent from all organs, except in the lungs and kidneys where a few caseous lesions could be found. The results of this series of experiments, consisting of 12 rabbits, showed some retardation in the spread of tuberculosis from a site of subcutaneous inoculation upon repeated intravenous injections of 0.25% ferric chloride. Further studies on this subject are being continued.

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[5] Menkin, V., *J. Exp. Med.*, 1931, **53**, 171.

6752

Reciprocal Relationship Between Ovaries and Anterior Hypophysis as Factor in Control of Lactation.*

WARREN O. NELSON.† (Introduced by Carl R. Moore.)

From the Hull Zoological Laboratory, University of Chicago.

Experimental evidence has been presented (Nelson,[1] Nelson and Smelser[2]) which has been interpreted as indicating the existence of a reciprocal relationship between the ovaries and the anterior hypophysis in the control of mammary gland development and lactation. This hypothesis, which is related to that of Moore and Price[3] regarding the control of sex rhythms, held that while the female sex hormones are responsible for the mammary gland development of pregnancy they also inhibit the secretion of the anterior lobe hormone responsible for lactation. With removal of the inhibition at parturition the hormone is secreted and lactation occurs. It is evident that this conception correlates at least most of the known facts relating to normal and induced lactation. Recently, Smith and Smith,[4] and Selye, Collip, and Thomson[5] have presented evidence which supports this hypothesis.

Supporting the earlier experiments with non-pregnant female and male guinea pigs are the following observations from a series of studies on pregnant animals. It will be seen that they offer important evidence in support of the reciprocal relationship concept.

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* This investigation has been aided by a grant from the Committee in Research in Problems of Sex of the National Research Council; grant administered by Prof. Frank R. Lillie.

† National Research Council Fellow.

[1] Nelson, W. O., *Anat. Rec.*, 1932, **54**, 51.

[2] Nelson, W. O., and Smelser, G. K., *Am. J. Physiol.*, 1933, **103**, 374.

[3] Moore, C. R., and Price, D., *Am. J. Anat.*, 1932, **50**, 13.

[4] Smith, G. V. S., and Smith, O. W., *Am. J. Physiol.*, 1933, **103**, 356.

[5] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 588.

1. The injection, into pregnant guinea pigs, of a potent anterior lobe extract‡ terminated pregnancy and induced lactation (8 cases).

2. Extirpation of the ovaries did not invariably lead to abortion (7 out of 21 cases went to term). Lactation occurred only after foetal expulsion whether abortive or at term.

3. Surgical removal of the the entire pregnant uterus did not cause lactation unless the anterior lobe extract was administered (11 cases).

4. Surgical removal of the uterus and the ovaries did induce lactation (5 cases).

5. Surgical removal of the ovaries and the fertile uterine horn only, in cases of unilateral pregnancy, resulted in lactation (4 cases).

6. Lactation did not occur when the ovaries and embryos were removed, leaving the placentae intact, during the period of placental retention, but did occur following expulsion of the placentae (4 cases). Autopsies of animals failing to lactate following natural, although abnormal, deliveries has revealed the retention of placental tissue. Lactation may be induced in such animals by administration of anterior lobe extracts.

7. Lactation was inhibited in normal parturient females by theelin administration (5 cases). However, if an anterior lobe extract was administered simultaneously with the theelin, lactation occurred (3 cases).

These experiments further confirm the original contentions and are especially pertinent as they deal with the pregnant animal. They indicate that, in the guinea pig at least, a dual control is exercised over the hypophysis during pregnancy by the ovaries and the placenta. The suppressing factor of the placenta is believed to be oestrin since, if abortion does not occur, the nipples continue to develop following ovariectomy. The nature of the ovarian hormone active in these animals is less clearly defined since the evidence at hand indicates that mammary development ceases when the pregnant uterus is removed. However, certain considerations indicate that the hormone of the corpus luteum, too, may be involved in the suppression of the hypophysis.

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‡ The sheep pituitary extract and theelin were generously supplied through the kindness of Dr. Oliver Kamm, of Parke, Davis and Co.

6753

Comparison of Concentration of Glucose in the Stomach and Intestine after Intragastric Administration.

I. S. RAVDIN, C. G. JOHNSTON* AND P. J. MORRISON.
(Introduced by S. S. Goldschmidt.)

From the Laboratory of Research Surgery, University of Pennsylvania.

The absorption of glucose from the intestinal tract has long interested investigators of carbohydrate metabolism. The investigations have been, in the main, performed by one of 2 methods. In the one, isolated segments of the small intestine have been used as an acute experiment in anesthetized animals, or as Thiry or modified Thiry loops in unanesthetized animals. In the other, either the whole or a large part of the gastro-intestinal tract has been used in anesthetized or unanesthetized animals. Data obtained have been published under the general heading of intestinal absorption, and certain generalizations have been drawn by one group which were often not substantiated by another. Comparisons of data were frequently made when the experimental methods were entirely different. Even when similar methods were used the data of different investigators often did not agree.

The effect of anesthesia, of different segments of the small intestine, the condition of the animal, and probably more than these the effect of the stomach on any solution of sugar placed in it, have frequently not been taken into consideration in evaluating the data obtained by the different groups of investigators.

Data which we had obtained from isolated jejunal loops, prepared by the method of Johnston[1] did not substantiate Cori's[2] generalizations, that regardless of concentration glucose solutions were absorbed at the same rate. Nor did we find a straight line relationship between time and the amount of glucose absorbed, or any correlation between body weight and the glucose absorbed from the jejunal segment. We were, therefore, led to conclude that, at least, as far as the isolated jejunal loop of the unanesthetized dog, Cori's generalizations were not correct.

We were aware, however, that while we were using only a single segment of a physiological unit, Cori had used the unit as a whole. Whether this difference in the experimental method might, at least in part. account for the contradictory results remains to be established. Cori[2] noted that significant amounts of fluid were found in the stomach at the conclusion of some of his experiments. Since our experiments were done on the dog we believed it important to ascertain what effect the stomach of this animal may have on any solution of glucose which is introduced into it.

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* Harriet M. Frazier Fellow in Research Surgery.

[1] Johnston, C. G., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 193.

[2] Cori, C. F., *J. Biol. Chem.*, 1925, **66**, 691.

Unanesthetized healthy dogs weighing from 11 to 15 kg. were starved for 24 hours before the experiment. A stomach tube was introduced and a measured amount (200 cc.) of a solution of glucose (Bacto-dextrose, Difco) of varying concentrations, which had been warmed to body temperature, was introduced. When this had entered the stomach a small amount of water (10 to 15 cc.) was used to rinse the flask which contained the glucose and this wash was then placed in the stomach through the tube.

The animals were under constant observation during the period of the experiment which was in each instance, one hour. If any regurgitation or vomiting occurred at any time from the introduction of the glucose solution to the time of completion of the experiment the animal was discarded.

About 2 minutes before the completion of the experiment the animals were rapidly anesthetized by the intravenous injection of 50 mg. of sodium amytal per kilo of body weight. We did not encounter any vomiting during this phase of the experiment nor were the animals ever excited.

The abdomen was rapidly opened and a Spencer-Wells forceps placed on the esophagus just below the diaphragm, 2 at the pylorus, and one on the small bowel at the ileocecal junction. These sections were rapidly removed and the contents collected in separate receptacles. The segments were not washed out since we were interested primarily in the concentrations of the solutions in the 2 segments and not in the absolute amounts of glucose present.

The amount of fluid in each container was measured and the glucose determined by the method of Benedict.[3] No correction was made for the reducing substances normally present in the stomach or small bowel, since in previous experiments we had determined that the amount present in the small intestine was negligible.

Results. The data obtained (Table I) demonstrate in a striking manner the rapidity with which water is drawn into the stomach, thus diluting the sugar solution which the stomach contained. Though 215 cc. was the maximum amount of fluid introduced into the stomach more than 500 cc. have been removed at the end of an hour. More striking is the uniformity of the concentrations of glucose recovered from the small bowel.

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[3] Benedict, S. R., *J. Biol. Chem.*, 1911, **9**, 57.

TABLE I.

Changes in the Concentration of Glucose.†

Dog	Time	Conc. Glucose in	Conc. Glucose out (Stomach)	Conc. Glucose out (Small Intestine)
No.	hr.	mg. %	mg. %	mg. %
762	1	3.5	2.3	—
789	1	6.0	3.7	—
793	1	10.4	6.3	3.5
788	1	14.9	7.4	3.3
792	1	19.9	10.5	3.6
766	1	20.0	5.0	2.6
808	1	20.2	5.9	2.9
790	1	23.6	9.8	2.4
794	1	27.0	10.8	3.6
809	1	50.0	17.9	5.3

† 200 cc. of solution were used in each experiment.

The concentrations recovered from this portion of the intestine are of such an order that even had equal amounts of these concentrations been placed in an intestinal loop, no differences in the rate of absorption may have been demonstrable. Since, however, the segments removed were not washed and since the precautions necessary, had total amounts of glucose been our objective, were not taken, we will include no data on the rate of absorption of glucose in this paper.

There can be little doubt from the data presented that where the gastro-intestinal tract is used as a physiological unit for studying the absorption of glucose it is not possible to draw any inferences on the rate of absorption from the small intestine, had only this portion of the gut been exposed to solutions of widely varying concentrations.

The changes in concentration of the glucose solution which remains in the stomach due to the rapid mobilization of water, tend to bring the concentrations into a closer relationship within a period of only one hour. A longer period of time would probably result in an even closer equalization. Furthermore, as the stomach contents escape through the pylorus into the duodenum the mechanism tending to equalize the concentrations must continue to be active, since in one animal we have observed the following changes in concentrations one hour after the introduction of a 23.6% solution—stomach 9.8%, duodenum 3.8%, jejunum and ileum 2.4%.

It is not possible at this time to state that the mechanism of dilution is the sole factor explaining the differences in the results which are obtained when the entire gastro-intestinal tract, or only a segment of this is used. The rapidity of emptying of the stomach and the motility of the small intestine may also be factors. When a jejunal segment is used no relationship exists between body weight and the rate of absorption of glucose while Cori[8] and Trimble, Carey and Maddock[9] have found that such a relationship exists when the gastro-intestinal tract is used as a physiological unit. This relationship must depend upon the rate of passage of the glucose solution into the small bowel or to differences in the rate of absorption from that portion of the intestine which acts as the absorbing membrane.

Conclusions. Data have been presented which demonstrate the rapidity with which solutions of glucose of varying concentrations when placed in the stomach of the dog are changed, so that when the solutions reach the small intestine the variations in the concentrations are of a small order. These findings may in part explain the differences in the rate of absorption when the gastro-intestinal tract is used as a physiological unit and when only a single segment of this unit is utilized.

6754

Bloodless Method for Recording Respiration and Quantitative Determination of Alterations of Sensation in Small Animals.

ARTHUR D. HIRSCHFELDER AND ALFRED H. RIDGES.

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Schmitz and Loevenhart[1] published a method for judging the onset and duration of anesthesia in the sciatic nerve of rabbits by the disappearance of changes in respiration when the distal portion of the nerve was stimulated. They recorded the respiration with a tambour attached to a cannula introduced into the trachea.

In order to study anesthesia (general, local and spinal) analgesia and hyperalgesia in small laboratory animals, without performing surgical operations, we have devised the apparatus shown in Fig. 1.

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[9] Trimble, H. C., Carey, B. W., and Maddock, S. J., *J. Biol. Chem.*, 1933, **100**, 125.

[1] Schmitz, H. L., and Loevenhart, A. S., *J. Pharmacol. and Exp. Therap.*, 1924, **24**, 159, 167.

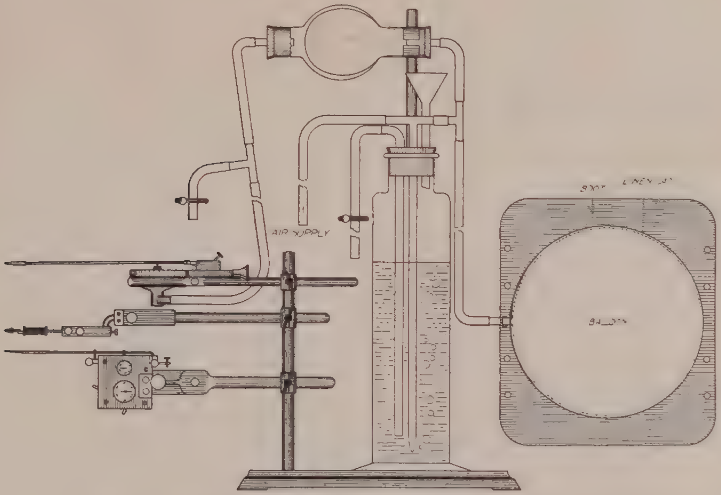

Fig. 1. Apparatus for bloodless registration of respiration.

The respiration is recorded using a modified blood pressure cuff which is laced around the chest and abdomen and connected with a tambour in the manner described below. Ordinary platinum electrodes are applied to the shaved skin (moistened with 0.9% NaCl) over the area to be tested. The stimulus is furnished by induction shocks from a Harvard induction coil, either faradic or break shocks being used. The coil distance is noted, so as to furnish a fairly quantitative measure of the stimulus.

For purposes of comparison, the threshhold of sensation can be taken as the minimal stimulus which causes a definite change in amplitude or rate of respiration.

The elastic sac of the respiration cuff for rabbits consists of a small toy balloon 18 cm. in diameter, covered with a linen sac (Fig. 1). The sac is placed inside of an ordinary tire boot through which a hole is made for the tube leading to the recording apparatus. The tire boot bears lacings and is applied to the animal's chest and abdomen. The rubber sac is connected by a rubber tubing and a T tube whose one branch inflates a rubber condom enclosed in a glass bulb. The other branch of the T tube is connected with a pressure bottle in which the pressure is kept constant by a slow stream of compressed air provided with a water valve maintaining about 15 cm. H_2O pressure as shown in the figure. Respiration movements are transmitted by pulsations of the condom to the air space in the glass bulb surrounding it and from this through a rubber tube to a recording tambour. The purpose of the air valve in the pressure system is to insure a rapid return of the condom to its original volume after the animal moves or breathes violently.

Similar cuffs for recording the respiration of rats, guinea pigs, and probably mice, can be made by using 2 thicknesses of adhesive plaster plastered together, and provided with laces at opposite edges.

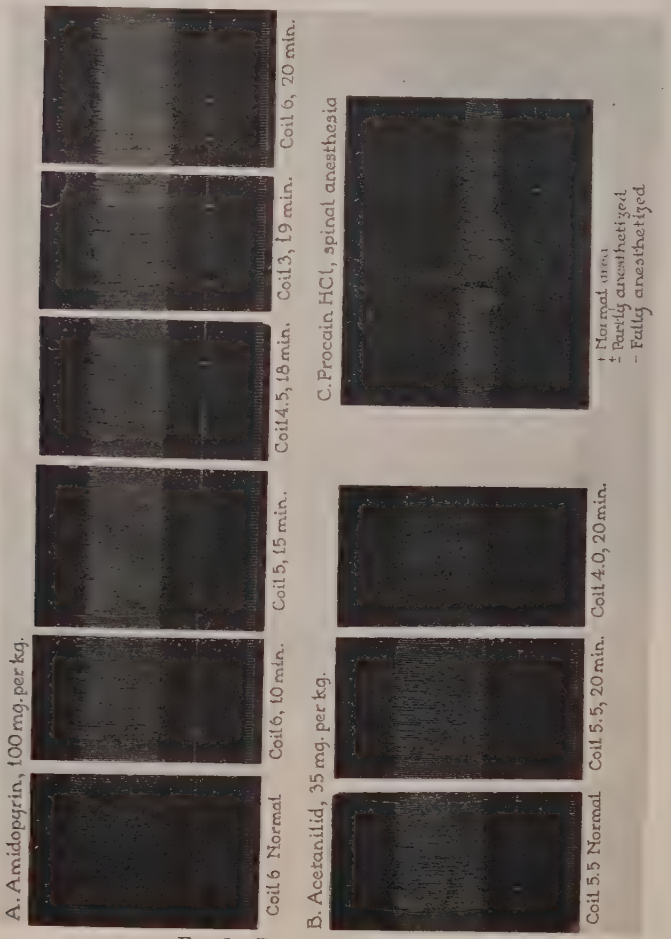

FIG. 2. Respiratory tracings.

A. Effects of amidopyrin at various intervals after injection and the coil distances of the stimuli used.

B. Effects of acetanilid.

C. Effects obtained in spinal anesthesia, when stimuli at same coil distances were applied over three different skin areas.

For such cuffs children's small toy balloons or finger cots provide the elastic sac. The pressure and recording apparatus is the same as for rabbits, the pressure in the cuff being adjusted to give the desired amplitude of recording.

By this method we have been able to study quantitatively the effects of analgesic drugs.

100 mg. amidopyrine (5% aqueous solution) per kilo subcutaneously raised the threshhold stimulus for brief faradic shocks with a Harvard induction coil from a normal of 6 cm. coil distance to 4.5 cm. (Fig. 2a). 35 mg. per kilo acetanilid raised it from 5.5 cm. coil distance to 4.75 cm. (Fig. 2b). Neither of these drugs caused any definite narcosis.

On the other hand, Hirschfelder and Cunningham[2] using the same method have found that 52 mg. morphine sulphate per kilo raises the threshhold of respiratory response from 6 cm. coil distance to 3 cm. and 50 mg. sodium amytal per kilo, raised it from normal of 6 cm. to only 5 to 5½ cm., although the animals were definitely soporous.

These findings harmonize well with the clinical observation that though the coal tar antipyretics and morphine relieve pain, the barbitals, sulphonal series, and most synthetic soporific drugs induce sleep but do not allay pain.

We believe that this method of testing affords the first satisfactory method for testing analgesic action quantitatively in laboratory animals. We have also used a similar method for determining sensitivity to pain in human beings, applying the electrodes to the mucous membranes on the inner aspect of the lip and determining both the stimulus which just produced a pain sensation and the stimulus at which the painful sensation became unbearable. Experiments on a small number of medical students indicated that an 0.6 gm. dose of acetphenetidin by mouth decreased both these stimuli by 0.5 to 1.0 cm. coil distance, *i. e.*, definitely increased the threshhold stimulus.

Our method is particularly well adapted for testing the onset and duration of local and spinal anesthesia. Rose[3] has used the minimal stimulus from a Harvard induction coil which just causes movement for testing local anesthesia with intracutaneous injections in the guinea pig. For this purpose our method furnishes a much more delicate index. We have also found it very satisfactory for testing the onset, degree, duration, subsidence and distribution of spinal anesthesia in rabbits (Fig. 2c). This subject is being investigated by Bieter and Ridges.[4]

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[2] Hirschfelder, A. D., and Cunningham, R. W., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 866.

[3] Rose, C. L., *J. Lab. and Clin. Med.*, 1929, **15**, 128.

6755

Variability Due to Technique of the Sedimentation Index.

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The striking variation in sedimentation rate as between individuals has led us to devote considerable attention to an analysis of this phenomenon. Studies of the influence of the technique employed and the interrelationships of the results by these techniques are proving fruitful in revealing important biological measures. Discussion of these results is being reserved until more extensive data can be accumulated, but it seems advisable at this stage to publish a note on the trustworthiness of a single determination of the sedimentation index.

Of fundamental importance at all times in the interpretation of the data of observation or experimentation is a knowledge of the variability to be expected in replicate measures of the same type. Not infrequently the worker in the fields of the so-called "exact sciences" is able to estimate *a priori* and from theoretical premises the error attaching to any method. Such estimates cannot, of course, be accepted as valid until verified in practical work, a fact which is all too often neglected. The biological worker can rarely follow such a procedure, if for no other reason than that the organism itself contributes an amount to the variability which can only be determined by the critical mathematical analysis of actual results.

Many techniques of making the sedimentation test have been in use during the past 10 years, but since the Cutler,[1] the Linzenmeier,[2] and Westergren[3] methods have had the greatest number of followers, the present discussion will be limited to these methods.

The extent to which any single measure, or average of replicate measures of blood sedimentation is reliable as a basis of generalizations must be dependent basically upon a precise determination of the variability of a large number of replicate determinations.

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[4] Bieter, R. N., and Ridges, A. H., unpublished experiments.

[1] Cutler, J., *Am. J. Med. Sci.*, 1926, **171**, 882; *Am. Rev. Tuberc.*, 1929, **19**, 544.

[2] Linzenmeier, G., *München med. Wchnschr.*, 1923, **120**, 1243.

[3] Westergren, A., *Am. Rev. Tuberc.*, 1926, **14**, 94.

To obtain information regarding the accuracy of the Cutler (5 cc.) technique, which we have favored because of its convenience in certain respects, 2 relatively large series of determinations were carried out on samples of blood as nearly homogeneous as possible and drawn at one time. The first, Series I, made use of horse blood. The very rapid sedimentation of the equine erythrocytes necessitated the filling of the tubes from an open vessel in which the fluid was constantly stirred. This led to an error of a range of ±2 mm. in filling the tubes to the zero mark. Also, it was exceedingly difficult to make precise readings rapidly at the beginning of the experiment and the observations at the 5 and 10 minute periods were rejected on this account.

Since some of the sources of error were inherent in the nature of the sedimentation of the blood used, the experiment was repeated upon a sample of human blood drawn from a healthy individual. These results comprise Series II. Using a pipette, the sedimentation tubes were filled exactly to the zero mark. The readings were all made by one observer. The sedimentation was read to the nearest mm. and the authors believe that the records are precise within that range of error.

In establishing the variability of any one sedimentation technique for general purposes, it would seem desirable to include in the study results from a number of subjects of a wide range of sedimentation indices. Accordingly, for a comparative analysis of the accuracy of the determinations by each of the 3 methods, sedimentation rates were determined *in duplicate* for each of 45 young women attending the preliminary nursing course at the University of Minnesota, all apparently in good health. For this group, comprising Series III, the Cutler 1 cc. technique was employed, the Linzenmeier and Westergren methods being followed according to the original specifications.

As a measure of dispersal or "error" in magnitudes quantitatively measurable, the standard deviation, σ, enjoys an unchallenged popularity after the many decades of vigorous analysis in which it has been employed. Accordingly, the error inherent in the sedimentation techniques being studied herein will be measured by that statistic as defined originally by Karl Pearson. For Series I and II of the present data, the application of the well-known formula for the standard deviation is straightforward and presents no difficulty. In Series III, however, where determinations are available in duplicate only, σ may be evaluated most directly by employing another technique. The differences between duplicate measures provide a series of ranges for samples of 2, and the mean range may readily be computed. The equation

$$\sigma = \frac{\text{mean range}}{1.12838},$$

where the denominator is drawn from tables by Tippett,[4] may then be employed for evaluating the required standard deviation.

The form of distribution of error has not been investigated for the data under consideration because of the inadequacy of the number of cases for such purposes. Accordingly the hypothesis of a "normal" distribution of error will be made. This hypothesis has been tested by one of us[5] on extensive data of a physico-chemical nature, and therein has been found justifiable. Theoretically, the normal curve is of infinite range. For practical purposes it is necessary to consider its range as limited by ordinates bounding, say, the central 98% of the total area. Such ordinates occur at $\pm 2.3263\ \sigma$ from the mean, and in the following discussion "range of error" will be defined accordingly.

Results. Series I. The series employing equine blood provided information that is, from certain points of view, merely incidental to the main task, which must center about blood from human subjects. Nevertheless it is of interest to note the statistics of sedimentation obtained for this type of vascular fluid.

The very fast settling of the red corpuscles will be noted from the summary of results presented in Table I. The standard deviations, measuring the error inherent in the method, do not give any significant evidence of real differentiation at the various time intervals. Accordingly, the average "variance" (σ^2) may be accepted as the most reliable basis for the calculation of the standard devia-

TABLE I.

Statistics for 49 replicate determinations of sedimentation for equine blood (Cutler 5 cc. method).

Time Interval	Mean mm.	σ mm.	Time Interval	Mean mm.	σ mm.
min.			hr.		
15	23.45	1.09	1	27.59	1.01
20	24.96	0.90	2	28.51	0.95
25	25.84	0.93	6	29.18	1.04
30	26.10	0.95	18	29.43	1.01

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[4] Tippett, L. H. C., *Biometrika*, 1925, **17**, 364.

[5] Treloar, Alan E., *Cereal Chem.*, 1932, **9**, 449.

tion of replicate determinations, giving $\sigma = 0.99$ mm. Correcting for the error of filling the tubes (range $= \pm 2$ mm.), we find a new value for σ of 0.49 mm. It is of interest to compare this value with those arising from Series II and III.

Series II. Passing to the series of 50 determinations on the one sample of human blood (Table II), a sharp contrast will be noted in the mean rate of sedimentation. The sedimenting corpuscles take 6 hours to reach a level attained by the red cells of the horse blood in less than 15 minutes. However, the standard deviations of the replicates at each interval of time approximate to the value of 0.49 mm. secured for Series I. Securing the average value as indicated above, we find the standard deviation of replicated determinations for human blood to be 0.48 mm.

TABLE II.
Statistics for 50 replicate determinations of sedimentation for human blood (Cutler 5 cc. method).

Time Interval	Mean mm.	σ mm.	Time Interval	Mean mm.	σ mm.
min.					
10	2.16	0.46	60 min.	10.54	0.50
15	2.94	0.51	90 "	14.32	0.47
20	3.64	0.52	2 hr.	16.36	0.48
25	4.48	0.48	3 "	18.78	0.41
30	5.22	0.50	4 "	20.56	0.49
35	6.16	0.54	6 "	23.02	0.37
40	7.02	0.47	18 "	26.20	0.40
45	7.96	0.49			

Series III. The standard deviations arising from application of the method of ranges, as derived from the duplicate analyses by 3 techniques on the 45 women subjects, are given in Table III. The progressive increase in σ for the successive time intervals in the Linzenmeier tubes is without question statistically significant. Although not so orderly, the same trend is apparent for the Westergren

TABLE III.
Standard deviations of the errors of the Cutler (C), Westergren (W), and Linzenmeier (L) techniques, based on 45 duplicate tests.

Time Interval	Standard Deviation* C	W	L
hr.			
½	0.53±.06	0.26±.03	0.47±.05
1	0.61±.07	0.35±.04	0.59±.07
2	0.41±.05	0.32±.04	0.71±.08
6	0.55±.06	0.87±.10	0.97±.11
16-18	0.45±.05	1.22±.14	1.08±.12
Average	0.51±.03		

* ± standard error in each case.

technique. In the Cutler series, however, there is no such trend, the individual standard deviations deviating by random errors only from the average value of 0.51 mm. Thus the results by the Cutler (1 cc.) method on the heterogeneous blood samples are in excellent agreement with those arising in Series I and II from the homogeneous samples, and the weighted average σ of 0.50 must surely give a highly dependable estimate of the variation due to technique in blood sedimentation readings by the Cutler methods.

Considering now the Westergren and Linzenmeier* values, it would appear to be a reasonable deduction that, for the one hour readings, the Westergren method is somewhat more consistent than the Cutler, and the Linzenmeier less accurate in replication. The latter is clearly more open to question than the former. For the half-hour readings, the Westergren method is without doubt more consistent in its results than either of the other 2, while after several hours the Cutler readings are unquestionably more accurate than those arising from either the Westergren or Linzenmeier techniques.

We reserve for later consideration the relative merits of the half-hour or one hour readings for the measurement of the sedimentation characteristics of human blood.

Conclusions. The Cutler methods of blood sedimentation tests show consistent accuracy at all periods of time so far as errors of measurement are concerned. Replicated tests on the same sample of blood may be expected to vary over a range of 2.3 mm. in 98% of such tests, provided the error is of the type described by the "normal law of errors".

The Westergren method shows higher accuracy than the Cutler method at the half-hour period of sedimentation, the error increasing steadily with increase in the time interval until it is approximately double that of the Cutler at 16 hours.

The Linzenmeier technique appears to have an error equal to or greater than the Cutler according as the time of sedimentation is short or long, so far as absolute fall of the red-cell column is concerned. The method of accumulation of the data considered herein did not permit of a study of sedimentation time as ordinarily recorded for the Linzenmeier method. Such a study, if made, would have had little comparative value in the present connection.

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* We are considering the Linzenmeier method here in terms of distance of fall as for the other techniques, and not in terms of time taken to reach the 18 mm. mark.

6756

A Method for the Study of the Rhythmic Property of the Human Heart.

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In the normal heart several centers of rhythm formation are potentially active, but the sinus node, which generates the impulse most rapidly, sets the pace of the heart. Observations on experimental animals indicate that there is considerable variation in the rhythmic efficiency of other portions of the heart. Cohn, Kessel and Mason,[1] after removing the sinus node in dogs, found a transitory quiescence of the heart varying from 4 seconds to 3 minutes, the interval being less than 30 seconds in most instances. The period of the quiescence was interpreted as representing the time required for some other portion of the heart to take on the pacemaking function. Rothberger and Winterberg[2] demonstrated in dogs that depression of the sinus node by stimulation of the right vagus nerve usually resulted in cardiac standstill. After eliminating the sinus node by vagus stimulation these observers studied the influence of various procedures in inducing new centers of impulse initiation. Thus they found that upon stimulation of the left sympathetic nerve, cardiac standstill was prevented by the development of new rhythmic centers in the ventricles. The addition of barium chloride or calcium chloride further increased the automaticity of the ventricles.

The purpose of this report is to point out that the above experiments may be reproduced in man, permitting a study of the rhythmic property of the human heart and also a direct method for the investigation of the action of drugs on this function. Utilizing the carotid sinus reflex of Hering,[3] pressure over the right carotid artery in the neck induces a reflex stimulation of the vagus nerve. In most individuals the degree of vagal stimulation is such that only a moderate slowing of the heart occurs. In some subjects, most frequently in elderly individuals, a very active vagus effect is obtained and the function of the sinus node may be entirely eliminated for a comparatively long period.[4]

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[1] Cohn, A. E., Kessel, L., and Mason, H. H., *Heart*, 1912, **2**, 311.

[2] Rothberger, C. J., and Winterberg, H., *Arch. f. d. ges. Physiol.*, 1911, **142**, 461.

[3] Hering, H. E., *Die Karotissinus reflexe auf Herz und Gefasse*. Dresden and Leipzig, 1927, T. Steinkopff.

In a series of electrocardiographic observations applying this reflex, an exaggerated reaction was obtained in 34 cases in which the heart was deprived of its normal pacemaker for intervals varying from 5 to 15 seconds. In 6 cases the elimination of the normal pacemaker was followed promptly, or within a few seconds, by an ectopic rhythm of ventricular origin. (Fig. 1.) In 25 instances the carotid sinus stimulation induced a prolonged standstill of the heart, as there was no spontaneous tendency for the ventricles to take on the pacemaking function. (Fig. 2-a.) The cardiac activity was resumed only when the sinus node again became active. In many cases the reflex was carried out repeatedly over a long period with a consistent response. In 3 instances the reaction was variable, resulting in cardiac standstill on one occasion and in a ventricular rhythm on repetition of the experiment. These observations indicate in most instances a relatively low efficiency of the secondary rhythmic centers of the human heart.

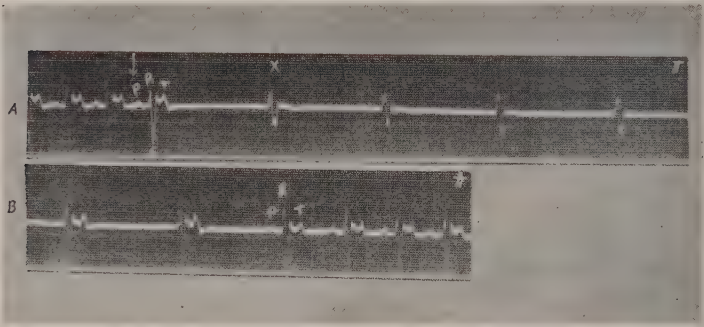

FIG. 1.

A and B form a continuous strip. Ventricular rhythm starting at "x" following pressure on right carotid sinus (arrow). P–P interval (inactivity of sinus node) is 14 seconds.

The subjects who show no tendency for a spontaneous formation of ectopic rhythmic centers permit a direct and objective study of the action of drugs on the rhythmic function of the human heart. The method consists of a control electrocardiogram recording the effect of the right carotid sinus pressure. (Fig. 2-a.) The drug is then administered and after suitable intervals, records are taken again, observing the response to stimulation of the carotid sinus.

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[4] Nathanson, M. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1037.

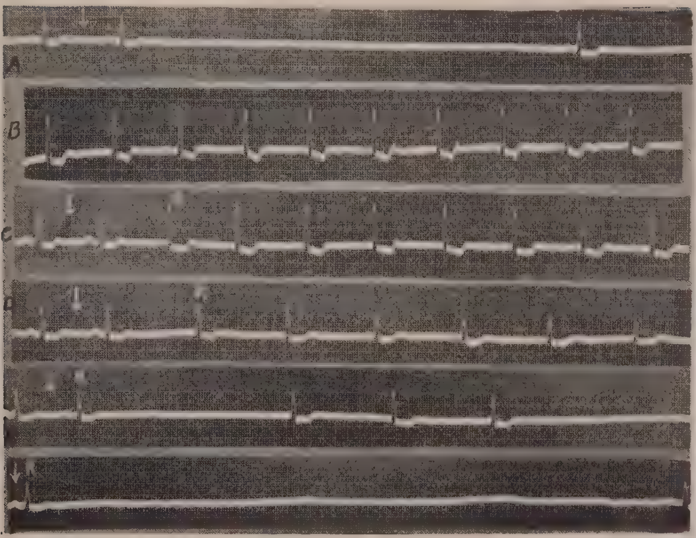

FIG. 2.

Strip A shows standstill of 7.6 seconds on stimulation of the right carotid sinus (arrow). Lower strips taken at intervals following 1/20 mg. epinephrin intravenously. Standstill abolished by development of an idioventricular rhythm. Lowest strip shows disappearance of epinephrin effect, 15 minutes after injection.

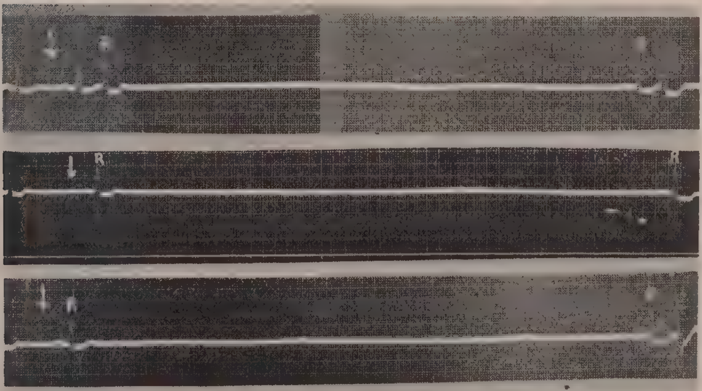

FIG. 3.

Effect of 50 mg. of ephedrine sulphate intravenously. Upper strip before and lower strips 5 and 15 minutes after the injection. No effect in stimulating secondary rhythmic centers.

The indication that a drug effectively increases the rhythmic function of the heart is the abolition of the cardiac standstill by the formation of new rhythmic centers. Figures 2 and 3 illustrate the effect of 2 chemically related substances, epinephrin and ephedrin. Epinephrin actively increases the impulse-initiating property of the ventricle as evidenced by the formation of a secondary rhythmic center near the auriculo-ventricular node. Ephedrin is entirely ineffective. The effect of epinephrin was studied in 8 subjects and the drug abolished the cardiac standstill in all cases by inducing a ventricular rhythm. It is possible by this method to study accurately the time of onset, duration and intensity of the effect. It was also apparent from the results of varying doses of epinephrin that the rate and site of the ectopic pacemaker may be utilized for quantitative studies. Observations with a large series of drugs are in progress and will be reported in detail.

6757

Improved Single Injection Method for Rapid Diagnosis of Early Pregnancy from Urine.

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A modification of the Ascheim-Zondek method was reported for the rapid diagnosis of early pregnancy.[1] The luteinizing hormone was concentrated in samples of urine and injected in 2 doses into immature female rats which were autopsied within 36 hours. A total of 265 specimens collected up to that time was diagnosed without error.

During the past 2 years I have attempted to shorten the time required for diagnosis and to use immature female mice as well as rats in performing the test. Notwithstanding the preference expressed in certain quarters for the rabbit as a test animal in the diagnosis of pregnancy by the Friedman[2] technic, this method does not appear to offer any special advantages. If greater accuracy can be obtained by another method and the time required for diagnosis be equal to that claimed for the rabbit intravenous technic, the advantages attending the use of small animals become quite obvious.

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[1] Eberson, F., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 407; *J. Am. Med. Assn.*, 1931, **96**, 2176.

[2] Friedman, M. H., and Lapham, M. E., *Am. J. Obst. and Gynec.*, 1931, **21**, 405.

The procedure shortens the time required for diagnosis to 18-24 hours instead of 36 hours. A smaller quantity of urine is needed and hence the amount of alcohol used in the purification process is reduced to one-third of that required in the older method. A single subcutaneous injection is sufficient. Alkaline urines are acidified slightly with acetic acid before concentrating. Preservatives such as boric acid or ether-tricresol do not interfere with the test.

To 50 cc. of recently voided morning urine are added 4 volumes of 95% ethyl alcohol and the mixture is placed in the ice-chest at a temperature of 4 to 6° C. for several hours, or until the precipitate has settled out. (This can be hastened by centrifuging the material promptly.) The clear supernatant fluid is decanted and the residue admixed with a small volume of alcohol is placed in a centrifuge tube and centrifuged briefly to pack the sediment. To the precipitate, freed from alcohol by decantation and drying in air current, 10 cc. of pure ether are added and the mixture agitated for 10 minutes. The ether is decanted and the sediment allowed to stand a few minutes in an air current to remove all traces of ether. Two cc. of sterile physiological salt solution are next added to the sediment which is thoroughly mixed and extracted. One to 2 hours or longer at the temperature of the ice-chest has been found satisfactory in the case of infrequent gummy residues. Usually a half hour suffices for the ordinary sediment, of which a major portion remains insoluble in the menstruum.

Immature female mice, 3 to 4 weeks old, are injected subcutaneously with ½ cc. of the saline extract. The mice are autopsied 16 to 18 hours later. Two animals are used, although there has been no mortality among the mice prior to autopsy. The macroscopic changes observed in the ovaries and tubes are clearly defined and the diagnosis of pregnancy is based upon the presence of "blood points" or visible corpora hemorrhagica and the typical congestion of ovaries and tubes. Not infrequently specimens of urine from patients in the menopausal age or with tumors in the reproductive tract produce changes which are readily differentiated from the genuine luteinizing reaction. Under such circumstances the ovaries are rarely enlarged or congested to the same degree and do not show hemorrhagic points. Indeed, the tubes are extraordinarily enlarged and edematous rather than congested, and the ovaries are often very small and colorless. This reaction, said to be due to the presence of Prolan A (follicle-ripening hormone), can be inhibited if desired by heating the hormone extract to 70° C. for 20 to 30 minutes before injection.

A series of 361 consecutive specimens has been studied without an error in diagnosis. The results are briefly summarized without attempting to give detailed clinical data.

TABLE I.
Results in 361 Cases.

	No.	Positive	Negative
Normal pregnancy	129	142	0
Abnormal pregnancy (a)	13		
Non pregnant patients	219	0	219
	361	142	219

(a) Ectopic, 7; tumor, 3; dead fetus, 1; incomplete abortion, 2.

TABLE II.
Normal Pregnancy.

Delay in menstrual period (days)	Number
None	3
0- 7	1
7-14	12
15-21	19
22-28	20
29-35	23
36-42	17
43-49	9
50-56	6
57-63	13
2½ to 8 months gestation	6
	129

TABLE III.
Abnormal Pregnancy.

	No.	Positive		No.	Positive
Ectopic pregnancy (b)	7	7	Dead fetus	1	1
Incomplete abortion	2	2	Tumor complication	3	3
				13	13

(b) 3 in this group had not missed the period due.

The results indicated that the majority of patients were in the early weeks of pregnancy. Approximately 43% fell into the group having either no missed period or a delay ranging from 1 to 4 weeks, and there were 31% with periods 4 to 6 weeks late, making a total of 74%. Three ectopic pregnancies were diagnosed in a group of 6 patients that had not missed a period, and 4 other ectopic cases occurred in a group with periods that had been delayed from 2 to 4 weeks. The large group of non-pregnant patients included 76 or approximately 35% with tumors of the reproductive tract.

6758

Pathways for the Secretory Fibers of the Salivary Glands in Man.

FREDERICK LEET REICHERT AND EDGAR J. POTH.*

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The innervation and mechanism of salivary secretion has been studied by many investigators, but completely satisfactory explanations of the various observations have not been reached partly because the innervations of the parotid, sublingual and submaxillary glands are not constant in different species of experimental animals or even in a single species. Furthermore, the fact that the innervation is not a simple one makes it all the more difficult to arrive at a definite and satisfactory understanding.

Howell,[1] speaking of the salivary glands of the dog, states as follows: "These glands receive their nerve supply from 2 general sources—namely, the bulbar autonomics (or cerebral fibers) and the sympathetic autonomics. The parotid gland receives its bulbar fibers from the glossopharyngeal or ninth cranial nerve; they pass into a branch of this nerve, known as the tympanic branch or nerve of Jacobson, thence to the small superficial petrosal nerve, through which they reach the otic ganglion. From this ganglion they pass (postganglionic fibers) by way of the auriculotemporal branch of the inferior division of the fifth cranial nerve to the parotid gland. The sympathetic autonomics pass to the superior cervical ganglion by way of the cervical sympathetic, and thence as postganglionic fibers in branches which accompany the arteries distributed to the gland. The bulbar autonomic supply for the submaxillary and sublingual glands arises from the brain in the facial nerve and passes out in the chorda tympani. This latter nerve, after emerging from the tympanic cavity through the Glaserian fissure, joins the lingual nerve. After running with this nerve for a short distance, the secretory (and vasodilator) nerve fibers destined for the submaxillary and sublingual glands branch off and pass to the glands, following the course of the ducts. The supply of sympathetic autonomics has the same general course as those for the parotid."

This is given as quite definite for the dog, but the arrangement is admittedly different for other species, and so might be far from true in man. There are probably 3 distinct sets of nerves to the salivary glands; the sympathetic, parasympathetic, and trophic fibers of Heidenhain. Therefore it would be quite normal to expect that these fibers entered the organs by different routes.

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* Fellow in Medicine of the National Research Council.

[1] Howell, W. H., 1926, IX ed., 755; W. B. Saunders Co., Phila.

In this investigation the rates of secretion of the parotid and submaxillary glands have been studied. The secretions were collected from human subjects in whom either the chorda tympani had been severed during mastoidectomy (sometimes with the facial nerve) or the ninth nerve had been cut intracranially. The secretions were collected by placing tared tampons over the duct openings of the glands, the tongue stimulated with lemon juice, and the tampons with the absorbed secretions reweighed.[2]

When the glossopharyngeal or ninth nerve is sectioned intracranially for tic douloureux there results unilateral anesthesia of the nasopharynx, soft palate, posterior pharyngeal wall and tonsillar region, loss of taste and sensation over the unilateral posterior third of the tongue. The only muscle innervated by this nerve is the stylopharyngeus, the paralysis of which cannot be detected.

Some authors[3, 4] have reported salivation during the paroxymal attacks of pain, and Hesse[5] has noted increased parotid secretion on the affected side, markedly decreased following section of the nerve. In 3 patients tested, who had one glossopharyngeal nerve cut intracranially where it is isolated from the other cranial nerves and readily divided without trauma to adjacent nerves, shortly after operation the collection of parotid secretion from the operated side was markedly diminished. This difference became smaller the longer the interval after operation.

Similar observations were obtained in the secretions of the submaxillary and sublingual glands, although the actual measurements were made of the submaxillary glands only. Starting with a ratio of 1 to 10 on the tenth postoperative day, it changed to 1 to 5 in 3 months.

It was surprising to find a markedly diminished submaxillary secretion shortly after section of the ninth nerve as the accepted theory has been that the secretory fibers pass from the brain in the seventh nerve and through the chorda tympani to the glands.

Through the courtesy of Drs. Sewall, Bacher, Cowan and Borden of the Department of Otolaryngology, the salivary secretions were collected from patients having radical mastoidectomies. In the cases examined the tympanic nerve (Jacobson's nerve), which is a branch of the ninth coursing in the middle ear and over the promontory, was believed not to be injured but almost invariably the chorda tympani was avulsed when the malleus and incus were removed. These patients not only showed a loss of taste on the ipsolateral anterior half of the tongue since it has been shown[6] that taste to the anterior half of the tongue courses through the chorda tympani, then through the seventh nerve to the brain.

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[2] Poth, E. J., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 977.

[3] Dandy, W. E., *Arch. Surg.*, 1927, **15**, 198.

[4] Reichert, F. L., *Surg. Clin., N. A.*, 1933, **13**, 193.

[5] Hesse, E., *Internat. Abst. S. G. and O.*, 1931, **52**, 114.

Four patients having loss of taste on the anterior ⅔ of the tongue following radical mastoidectomy had their salivary secretions collected 3 days to 4 months after operation. Not only was the submaxillary secretion markedly diminished (1 to 10 or 12) throughout the whole period without any change in the ratio but the parotid secretion was markedly diminished as well (1 to 3 or 5) and remained so for 4 months after operation.

From these observations it has been shown that after section of the glossopharyngeal nerve there is an immediate and marked diminution in secretion which, however, approaches that of the normal side by the third post-operative month. When the chorda tympani has been sectioned just distal to its exit from the facial canal and facial nerve, salivary secretion is markedly and permanently diminished.

One must conclude, therefore, that secretory fibers to the salivary glands leaving the brain by the glossopharyngeal nerve following its tympanic branch (Jacobson's nerve) into the middle ear where they cross in the region of the greater superficial petrosal

TABLE I.

Rates of salivary secretion following intracranial section of the glossopharyngeal nerve.

Patient	Operative Procedure	Time Postoperative	Parotid Operated side	Parotid Normal side	Submaxillary Operated side	Submaxillary Normal side
			gm.	gm.	gm.	gm.
R. S.	Section N IX	10 da.	0.25	2.20	——	——
	intracranially	10 wk.	2.7	4.5	0.10	0.90
	left. 12-10-32	13 "	1.05	1.20	0.10	0.55
E. H.	Section N IX	1 yr. 4 mo.	0.25	0.70	0.50	0.45
	intracranially				0.10	0.15
	left. 11-10-31					
J. M. S.	Section N IX	2 yr. 6 mo.	0.60	0.90	0.20	0.25
	intracranially					
	right. 7-3-30					

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[6] Lewis, D., and Dandy, W. E., *Arch. Surg.*, 1930, **21**, 249.

nerve to join the fibers of the seventh nerve in the region of the geniculate ganglion. Or, these fibers might be found to leave the ninth nerve via the anastomotic or communicating branch of the auricular branch of the glossopharyngeal to enter the seventh nerve distal to the chorda tympani because of the findings in Table II, Case E. In this case of peripheral Bell's Palsy with taste intact, a decrease of salivary secretion on the affected side is found.

Also, there is evidence that other secretory fibers course out from the brain through the seventh nerve. Thus the fibers as described from the ninth as well as those from the seventh are collected in the chorda tympani to be distributed to the parotid by way of the Otic ganglion and thence via the auriculo temporal branch of the fifth

TABLE II.
Rates of salivary secretion following section of chorda tympani in the tympanic membrane, and a case of peripheral Bell's palsy.

Patient	Operative Procedure	Post Operative	Parotid		Submaxillary	
			Operated side	Normal side	Operated side	Normal side
			gm.	gm.	gm.	gm.
A	Right Radical Mastoidectomy 11-24-32 Taste: Rt. Ant. 2/3 tongue gone	4 mo.	0.60 0.80	1.95 2.25	0.03	0.13
B	Bilateral Simple Mastoidectomy. Rt. facial palsy, not complete. Taste normal 2-20-33	11 days	1.45 (Rt.) 1.50 (Lt.)		0.05 (Rt.) 1.00 (Lt.)	
	Rt. Radical Mastoidectomy 3-8-33 Taste: Rt. Ant. 2/3 tongue gone	3 "	0.50 (Rt.)	1.25 (Lt.)	0.20 (Rt.)	0.65 (Lt.)
C	Rt. Radical Mastoidectomy 2-28-33 Taste: Rt. Ant. 2/3 tongue gone	4 "	1.0	3.60	None*	Abundant*
D	Rt. Radical Mastoidectomy 3-7-33 Taste: Rt. Ant. 2/3 tongue gone	5 "	0.55 0.50	1.00 1.10	0.05 0.03	0.25 0.25
E	Bell's Palsy Right Taste intact	5 wk.	0.60 (Rt.) 0.50 (Rt.)	1.10 (Lt.) 0.95 (Lt.)	0.15 (Rt.) 0.10 (Rt.)	0.25 (Lt.) 0.50 (Lt.)

* Gross observation. Not collected and weighed.

and to the submaxillary gland by way of the lingual branch of the fifth.

Summary. 1. The rates of secretion of the salivary glands in man after unilateral section of the ninth nerve and chorda tympani indicate peripheral pathways for secretory fibers to these glands other than those accepted. 2. Section of the ninth nerve intracranially causes marked temporary diminution of salivation with partial recovery involving parotid, sublingual and submaxillary glands. 3. Section of the chorda tympani in the tympanic membrane causes marked permanent diminution of salivation involving the parotid, sublingual and submaxillary glands. 4. Therefore, it is concluded that the salivary glands receive their secretory fibers from both the seventh and ninth nerves.

6759

A Simplified Technique for Quantitative Collection of Salivary Secretions of Man.

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Several methods have been devised for the collection of the salivary secretions of man, but most of them are either troublesome or inaccurate. The method presented here is applicable to the collection of the secretions of the parotid, sublingual, and submaxillary glands separately and simultaneously on both sides.

The method consists of applying pieces of tared absorbent material to the various duct openings, absorbing the secretions for the desired length of time, and then reweighing to nearest .05 gm. The quantity collected is given by difference. It is simpler to collect the secretions from the 3 pairs of glands separately, and the nasal tampons marketed by Johnson and Johnson are found to be the most satisfactory absorbent material.

To collect the secretions of the parotid glands, the floor of the mouth is packed off with dental rolls, and strips of tared nasal tampons about 4 cm. long are placed over the openings of Stenson's ducts. The strips will remain in place without support. They may be left in place until their thickness has doubled. This will require anywhere from one to 5 minutes depending on the rate of secretion and will represent a collection of one to 2 gm. of material. In collecting the secretions of the sublingual and submaxillary glands the parotids are packed off with dental rolls. Strips of nasal tampons are laid upon the ducts of Rivini bilaterally, and 2 other strips of the absorbent material separated by a piece of X-ray film and held by a clamp as illustrated in the accompanying cut, are placed on the openings of Wharton's ducts. Obviously, care must be taken to assure that the septum of X-ray film passes and remains between the 2 submaxillary ducts so that the secretion from each duct is absorbed on the proper strip.

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* Fellow in Medicine of the National Research Council.

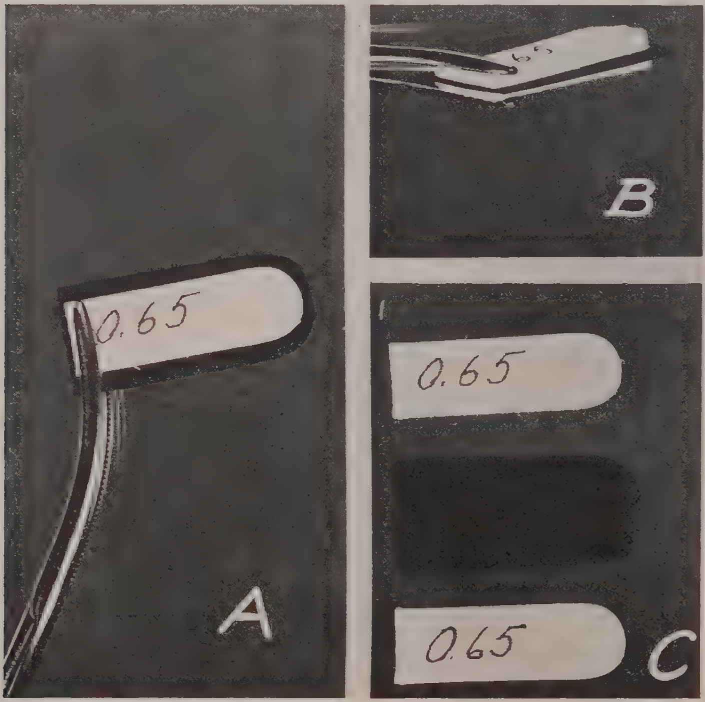

FIG. 1.
Arrangement for Collection of the Secretions of the Submaxillary Glands.
***A* and *B* show assembled materials; *C* shows tared nasal tampons (weight written on the absorbent material) and the septum of X-ray film.**

This method of collecting the salivary secretions is simple, rapid, accurate, and of little discomfort to the patient.

6760

Roentgenographic Visualization of Lymph Nodes and Vessels in the Human and in Laboratory Animals by Injection of Thorium Dioxide.*

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We have previously reported the roentgen visualization of lymph nodes and vessels in laboratory animals by subcutaneous and intraperitoneal injections of thorium dioxide (thorotrast).[1] We were able to visualize clearly many different lymph nodes and vessels in these living animals by means of the roentgen ray. Many of them are living in apparent good health over one year after injection.

After being convinced that laboratory animals were in no way harmed by the subcutaneous and intraperitoneal injection of thorium dioxide, we began our experiments on the human in an effort to visualize man's lymph nodes and vessels.

Case No. 1, a colored female, age 41, with an extensive carcinoma of the cervix and body of the uterus, was injected subcutaneously over the abdomen and into the tissues of the cervix. A radiographic examination made immediately after the injection showed the thorium dioxide in the subcutaneous tissues of the abdomen and also in the cervix. No ill effect from the injection was experienced by the patient and none observed by us.

The patient, again examined 2 days later, had not experienced any symptoms which might be attributed to the injection of thorium dioxide. A radiograph made at this time clearly showed 2 lymph nodes situated in the pelvis, both circular in shape, one large and the other small. The small node had absorbed the thorium in an irregular manner; one-quarter of this gland had failed to absorb any thorium, possibly as a result of metastatic cells blocking that portion, and showed as a dark area in contrast to the remaining portion of the gland which had absorbed thorium and was light.

Case No. 2, a colored female, aged 50, with an extensive carcinoma of the uterus, with metastasis, was injected intraperitoneally

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* Aided by a grant from the General Baking Company. Patients were obtained through the courtesy of Dr. S. H. Wills' service of the Flint-Goodrich Hospital, and also through the cooperation of Dr. A. W. Brazier, of New Orleans. We acknowledge with appreciation the cooperation given us by Dr. F. T. Jones, of the Radiological Department of the Flint-Goodrich Hospital, New Orleans.

[1] Menville, L. J., and Ané, J. N., *J. Am. Med. Assn.*, 1932, **98**, 1796.

with thorium dioxide and radiographed immediately, to ascertain if the thorium dioxide had entered the peritoneal cavity. The patient felt no ill effect from this injection. Two days later by another roentgen observation we demonstrated some of the abdominal and diaphragmatic lymph vessels, leading to the chest, and also several abdominal lymph nodes. At this examination the patient stated that, several hours after the injection of the thorium dioxide, she experienced slight abdominal cramp, which might have been caused by her illness; however, it had not been necessary to take medication for its relief, and she was now free from pain.

These experiments demonstrate that it is possible to inject thorium dioxide into the subcutaneous tissues and peritoneal cavity of man without producing harmful results; and that by such injections it is possible to visualize human lymph nodes and vessels by means of the roentgen ray.

In experiments already reported[2] it was shown that the absorption of thorium dioxide when injected intraperitoneally in rats was, first, by means of the abdominal lymphatics, then by the diaphragm and by the intercostal lymph nodes and vessels, terminating in the right and left lymphatic ducts.

Recently we reported[3] that when thorium dioxide is injected into the peritoneal cavity of dogs it is absorbed by the abdominal lymphatics, the diaphragm, the substernal lymph nodes, and the anterior and posterior mediastinal glands; also the substernal lymph vessels. These experiments clearly demonstrate that thorium dioxide is readily absorbed from the peritoneal cavity and that its route of absorption is upward.

In the experiments herein reported, dogs, rabbits, and rats were injected interpleurally with thorium dioxide. The dogs were injected under the fluoroscope, affording an unusual opportunity to observe the route taken by the thorium, which first quickly filled the different fissures on the side injected. The thorium was seen to leave the needle, and quickly and clearly fill the fissures and then follow the pleural route.

Though all of our animals were injected on the right side, in less than one hour after injection lymphatic vessels were demonstrated on both the right and left sides of the thorax and the diaphragm.

The first portion of the lymphatic system of the chest to be visualized after the interpleural injection was the substernal glands, less than one hour after injection.

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[2] Menville, L. J., and Ané, J. N., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 28.

[3] Menville, L. J., and Ané, J. N., read before Radiological Society of North America, Nov. 30, 1932. Not yet published.

The animals were not injured by the interpleural injection of thorium dioxide. Those first injected several months ago are in apparent good health.

These experiments demonstrate that a colloidal substance, such as thorium dioxide, when injected as reported, is first absorbed by the substernal glands, by the lymph nodes and vessels of the thorax and by the lymph nodes and vessels beneath the diaphragm. It is believed that substances other than colloidal in character, such as organisms, if injected into the pleura in a similar manner will be absorbed by the lymphatic system in the same way.

6761

Excretion of Phenol Red by the Aglomerular Kidney.*

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The fact that the aglomerular kidney can excrete phenol red was first reported by Marshall and Grafflin[1] for the goosefish, and by Marshall[2] for the toadfish. Marshall and Grafflin[3] have described the excretion of phenol red by the aglomerular kidney following the intramuscular injection of varying doses, and the relation between the amount excreted and the urine volume. Their experiments were conducted on summer toadfish. The excretion of the dye was determined for a period of 5 hours. Averaging their figures gives the following results on

a. Effect of varying the amount of phenol red administered:

mg. Phenol Red per kg. body wt.	% excreted 5 hr.	mg. excreted 5 hr.
5	17.5	0.9
50	3.8	1.9
100	1.6	1.6

b. Effect of variations in urine flow:

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*This work was made possible by a grant from the Graduate School Research Fund of the University of Minnesota.

[1] Marshall, E. K., Jr., and Grafflin, A. L., *Johns Hop. Hosp. Bull.*, 1928, **43**, 205.

[2] Marshall, E. K., Jr., *Am. J. Physiol.*, 1930, **94**, 1.

[3] Marshall, E. K., Jr., and Grafflin, A. L., *J. Cell. and Comp. Physiol.*, 1932, **1**, 161.

mg. Phenol Red administered per fish 200-400 gm.	Urine flow per kg. per 24 hr.	% excreted 5 hr.	mg. excreted 5 hr.
1.8	1.1	20.7	0.37
1.8	2.6	23.3	0.41
1.8	15.8	21.9	0.39

Thus, when the injected dose of phenol red was increased 10 times, the mg. excretion was only doubled, and with a further doubling of the dose the excretion remained practically constant. In the second group of experiments, when the urine flow increased 160% the phenol red excretion increased only 12% and lastly with a further increase in urine flow of 600% the phenol red excretion remained practically constant. This proves that for the aglomerular kidney the secretion of phenol red is neither proportional to blood concentration nor dependent upon urine flow.

Marshall[4] has also shown in the toadfish, that from a blood concentration of 0.6 mg. % of phenol red, the urine produced may contain as much as 250 times this concentration; whereas from a blood concentration of 19 mg. %, the phenol red concentration in the urine only reaches a figure 14 times that in the blood. This shows that with increasing the blood concentration the efficiency of the kidney is decreased.

As the dosage employed by Marshall and Grafflin was much higher than that used in the human, it was thought worthwhile to study the excretion of phenol red by the toadfish using doses comparable to the arbitrarily accepted 6 mg. dose for the human. This paper reports these experiments. As the urine flows of toadfish in this laboratory have been found to be low and extremely constant, the effects of varying urine outputs were not included. The results obtained following the intramuscular injection of varying quantities of phenol red completely confirm the findings of Marshall and Grafflin.[3]

The toadfish used in this study can, perhaps, best be called winter fish. They were shipped from Baltimore in October and November. In the laboratory, they were contained in aquaria filled with half strength artificial sea water (240 millimols NaCl per liter). The formula for the preparation of the sea water was obtained from McClendon, Gault and Mulholland.[5] The fish lived extremely well in the laboratory; none died. They were fed once per week on either fresh oysters from the shell, or canned oysters.

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[4] Marshall, E. K., Jr., *Am. J. Physiol.*, 1931, **99**, 77.

[5] McClendon, J. F., Gault, C. C., and Mulholland, S., Publication No. 251 of Carnegie Institution, Washington, 1917, 21.

The experimental procedure was to remove the fish from the aquarium with a net, weigh, tie off the urinary papilla, inject the phenol red intramuscularly dissolved in 0.4 to 0.6 cc. of water and then return the fish to the aquarium. The fish were out of water, for these steps, for only 2 to 3 minutes. Two hours later, the fish were again removed from the aquarium, sacrificed, and the urine removed from the bladder with a hypodermic syringe. In addition, the bladder was always washed out twice with small volumes of distilled water. The dye in the urine was then determined with a micro Duboscq colorimeter.

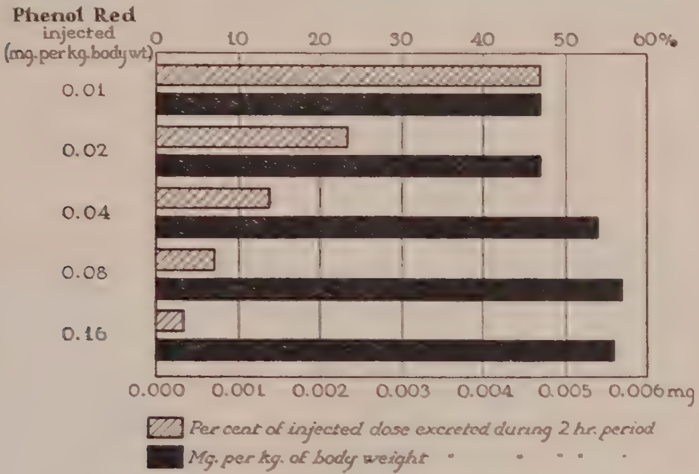

FIG. 1.

Excretion of Phenol Red by Aglomerular Kidney of the Toadfish.

Fig. 1 shows graphically the average % excretion and the average mg. per kg. of body weight excretion of phenol red for the 5 doses administered; namely, 0.01, 0.02, 0.04, 0.08, and 0.16 mg. per kg. Each time the injected amount of phenol red is doubled the % excretion is cut almost in half. On the other hand, the excretion in mg. per kg. of body weight remains constant or possibly tends to increase very slightly over the 5 dosages studied. Assuming uniform absorption of the dye from all injections, these results indicate that the secretion of phenol red by the aglomerular kidney is not dependent upon the blood concentration, and therefore does not agree with the ordinary concepts of filtration or diffusion.

Summary. When phenol red is injected intramuscularly into toadfish in doses of 0.01, 0.02, 0.04, 0.08 and 0.16 mg. per kg. of body weight, the % elimination from these doses is 47, 23.5, 13.9, 7.3 and 3.5, respectively. Based on body weight of the fish, this elimination amounts to 0.0047, 0.0047, 0.0054, 0.0057 and 0.0056 mg. per kg. Through this series, increasing the injected dose up to 16 times, increases the mg. per kg. elimination by not more than one-fifth. In other words the excretion remains practically constant.

6762

Modification of Skin Sensitivity to Neoarsphenamin in Rabbits Treated with Potassium Iodide and Bromide.

J. W. MU. (Introduced by C. N. Frazier.)

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It has been demonstrated[1, 2, 3] that a nonspecific positive luetin reaction can be produced in the human subject and animals by the administration of potassium iodide. The object of the present study was to ascertain whether or not potassium iodide and bromide are capable of exerting a similar influence on the skin hypersensitiveness to neoarsphenamin in rabbits.

This work included 2 experiments, involving 76 adult male albino rabbits. Twenty-four of these animals which had been inoculated with *Treponema pallidum* from 5 to 8 months previously, were distributed in both experiments and were all in the latent stage of the infection at the time of the experiments. The neoarsphenamin solution was prepared and the rabbits were shaved according to the methods described previously.[4] The brand of neoarsphenamin used was that prepared by Hoechst Company, Germany, and the ampoules employed were always from the same batch. The rabbits were sensitized and tested with 0.2 cc. of freshly prepared 0.15% neoarsphenamin in normal salt solution. The right flank of the animal was used for the sensitizing injection and the left for the testing injections, all being intradermal. The hyper-

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[1] Sherrick, J. W., *J. Am. Med. Assn.*, 1915, **65**, 404.

[2] Kolmer, J. A., Matsunami, T., and Broadwell, S., Jr., *J. Am. Med. Assn.*, 1916, **67**, 718.

[3] Kolmer, J. A., Immerman, S. L., Matsunami, T., and Montgomery, C. M., *J. Lab. and Clin. Med.*, 1917, **2**, 401.

[4] Mu, J. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 781.

sensitive reaction, which was manifested as redness and infiltration, was read 24 hours after injection, the size of the involved skin area ranging from 1 to 3 cm. in diameter.

In the first experiment, 42 rabbits were used and in the second one, 34 rabbits. Five weeks after the animals had been sensitized, they were tested for hypersensitiveness (first test). As shown in the accompanying table, the animals of each experiment were then divided into 3 groups, each of which included both hypersensitive and non-sensitive rabbits of about equal number. The first group of each experiment was used as control and the others as test groups. The test groups in the first experiment were injected intravenously with 10% aqueous solutions of potassium iodide and potassium bromide respectively. To determine the relative action of the individual ions of the drugs used in the first experiment, the test groups in the second experiment were treated intravenously with 10% aqueous solutions of potassium iodide and sodium iodide respectively. The chemicals employed in both experiments were given once a day for 3 days in daily doses of 0.02 gm. per kilo of body weight. Twenty-four hours after the final administration of the drugs, the animals of all groups in both experiments were tested again for hypersensitiveness (second test). The skin reactions were measured 24 hours after the second set of tests, and the animals were also observed for flare-up of the reactions from the first series of tests.

TABLE I.

Skin Reactions in Rabbits Receiving Sensitizing Doses of Neoarsphenamin and then Treated with Potassium Iodide, Sodium Iodide or Potassium Bromide.

Exp. No.	Group No.	No. animals	Hypersensitive animals on first test	Treatment	Animals showing increase of skin reaction	Non-sensitive animals becoming hypersensitive
I	1	14	4	No drug (control)	0	0
	2	14	5	Potassium iodide	3	7
	3	14	4	Potassium bromide	1	3
II	1	10	2	No drug (control)	0	0
	2	12	2	Potassium iodide	1	6
	3	12	4	Sodium iodide	2	5

As recorded in the table, the results indicate that potassium and sodium iodides intensified the skin hypersensitiveness to neoarsphenamin in 54% of the animals showing positive skin reactions on the first test, and produced well-marked cutaneous reactions in 66% of the animals which had not reacted to this arsenical before the administration of the iodides. The potassium and sodium iodides were about equally capable of exerting these influences. Potassium bromide was found to have a similar but much less effect. There was no appreciable difference in the response to the tests for sensitization after treatment with the iodides and bromide between the normal and syphilitic rabbits. No flare-up was noted at the site of the test conducted before the injection of the drugs under study. The control animals did not show any significant variation in their skin reactions after the first and second tests, thus ruling out the possibility that the changes observed in the rabbits treated with iodides and bromides were attributable to their skin reactivity being influenced by the preliminary testing injections. The observations reported here seem to support the conclusion that the modification of skin reactivity to neoarsphenamin in the animals of these experiments is to be ascribed to the action of the iodide and bromide ions and not to the potassium and sodium ions of the drugs used.

6763

An Optimal Diet in Promoting Nitrogen Gain in Nephrosis.

S. H. LIU AND H. I. CHU.

From the Department of Medicine, Peiping Union Medical College.

In the treatment of nephrosis Epstein[1] advocates a high protein diet, 150 to 200 gm., with low caloric intake. Peters and Bulger[2] from an investigation of the nitrogen metabolism in albuminuric, edematous patients conclude that it is possible to promote the storage of food protein and to cover the protein lost in the urine by allowing a moderate amount of protein in a high calory diet. The present study involves the determination of nitrogen balance and plasma proteins on 2 patients with nephrosis on diets which were varied in (1) total calories, (2) protein content and (3) proportion of animal and vegetable protein.

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[1] Epstein, A. A., *Am. J. Med. Sci.*, 1917, **154**, 638, and 1922, **163**, 167.

[2] Peters, J. P., and Bulger, H. A., *Arch. Int. Med.*, 1926, **37**, 153.

The first patient, 13 years old, had the nephrotic syndrome for 3 years before the study. He was in a state of convalescence at the commencement of this work which consisted of 47 periods of 4 days each, or 188 days. Weight at the start was 25.8 kg. With a constant protein intake of 50 gm. and 50% of animal protein, the caloric intake was raised (in steps of 280 calories) from 1750 to 2590 calories. The daily positive nitrogen balance, which averaged 3 gm., was not significantly altered. Progressive lowering of the caloric intake from 2590 to 1500 caused the nitrogen gain to lessen so that on 1500 calories the patient could hardly maintain nitrogen balance. He was then kept on 2310 calories, or approximately 75 calories per kg., while the protein content was raised. Feeding 75 gm. of protein resulted in an average daily nitrogen balance of 3.1 gm. as compared with 2.0 gm. on 50 gm. of protein intake, but further increase of protein intake to 100 gm. and then to 125 gm. did not promote any greater positive nitrogen balance. An optimal level of protein intake was apparently reached when 75 gm. or 2.5 gm. per kg. were fed. While the caloric and protein intake was constant at 2310 calories and 75 gm. respectively, the relative proportion of animal and vegetable protein was changed. No marked differences in nitrogen gain were observed, although it was slightly higher when greater proportion of animal protein was fed. Throughout the whole period of study plasma albumin stayed between 3.5 and 4.0% and total proteins between 5.5 and 6.5%.

The second patient, aged 35 years, presented a typical picture of nephrosis on admission. By the time he was studied his renal condition had so improved that he showed only slight pitting edema over the tibia and a trace of albumin and a few casts in the urine. Observations extended over 53 periods, or 212 days. Weight at the start was 51.1 kg. With his protein intake constant at 75 gm. and animal protein approximately 50%, diets containing 1800, 2260, 2660, 3250, and 3770 calories were successively given. The negative nitrogen balance of 2 gm. daily at 1800 calories became positive at 2260 calories and a maximum positive balance of 3.5 gm. daily was reached when 3250 calories were fed, and further increase in caloric intake to 3770 did not improve the balance. When the caloric intake was progressively decreased, the nitrogen gain decreased in proportion. It seemed that an optimum level of caloric intake in this case was established at 3250 or approximately 60 calories per kg. On this caloric intake the patient was given in succession 75, 50, 100, 125, 150 and 175 gm. of protein, 50% of which were of animal origin. A maximum nitrogen gain of approximately 4 gm. a day was reached at 100 gm. of protein or 1.8 gm. per kg. and higher protein feeding failed to promote further gain. With the caloric intake constant at 3250 calories and protein intake at 100 gm., the percentage of animal protein was varied from zero to 100%. In this instance 75% animal protein seemed to give better nitrogen gain than any other percentages. There was an increase of albumin from 2.3 to 3.0% and total proteins from 4.0 to 5.2% during the course of first 2 months of study, but after that they stayed fairly constant at these levels. The albuminuria, aside from its steady tendency to diminish as the study progressed, showed no significant variations associated with dietary changes.

In conclusion there seems to be an optimum level both for total calories and protein intake in order to secure maximum nitrogen gain. For an adult 1.8 gm. of protein and 60 calories per kg. seem optimal. The requirements are higher with children. The relative proportion of animal and vegetable protein in the diet does not seem important when both calories and protein are fed at optimal levels.

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Effects of Snake Venoms on Plants.

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With the exception of Mitchell[1] and Salisbury,[2] who made sporadic observations on the subject in nature, investigators have given very little attention to the effects of snake venoms on plants. In connection with phytopharmacological studies regarding the effect of various animal poisons on living seedlings, the writer made experiments with suspensions or solutions of snake venoms by dissolving small quantities of the dry scales in physiological saline. The method employed has been fully described.[3, 4, 5] Seeds of *Lupinus albus* are soaked for 24 hours in water and then planted in finely divided sphagnum moss. When seedlings have germinated and borne roots from 30 to 40 mm. long, they are carefully selected, "matched," and placed in upright, hard-glass test tubes containing a plant-physiological solution made up of equal parts of Shive's mixture and distilled water. The average growth of roots of 10 seedlings (in the dark at 20° C. for 24 hours) is determined. Dry scales of snake venoms were rubbed up with small quantities of physiological saline and then diluted with the plant-physiological solution mentioned above in various concentrations; and the growth of the *Lupinus albus* seedlings in such solutions was also determined. The relative inhibition of growth of seedlings in the solutions of venom, compared with growth of the controls, is the phytotoxic index and expressed in percentages. The control experiments, as well as the experiments with solutions of venoms, were all made with seedlings from the same crop, grown under exactly the same conditions of temperature, light, weather, etc. Such sets of experiments were made repeatedly so that the conclusions were definite, as will be seen from the figures in Table I. The venoms of the *Crotalus atrox, Crotalus exsul, Bothrops atrox, Ankistrodon piscivorus* and *Naja* (cobra) were studied.*

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[1] Mitchell, Smithsonian Institute, Contributions to Knowledge, 1860, **12**, 145.

[2] Salisbury, *J. Med.*, 1854, **13**, 837.

[3] Macht and Livingston, *J. Gen. Physiol.*, 1922, **4**, 573.

[4] Macht, *J. Pharmacol. and Exp. Therap.*, 1926, **29**, 461.

[5] Macht and Pels, *Arch. Dermatol. and Syphilol.*, 1931, **23**, 601.

It was found that all these venoms markedly *inhibited* the growth of the roots, thus indicating that they were toxic for plant protoplasm. Moreover, it was found that the emulsions of venoms rapidly decomposed on standing at room temperature (24°C.). Study of the table reveals the much lower toxicity of such emulsions after standing for 24 and 48 hours, respectively. The greatest toxicity for plants was exerted by the freshly prepared venom emulsions in which the seedlings were allowed to grow at a rather low temperature, 19°C. Another interesting finding was the considerable decrease in toxicity of the venom scales kept over a period of 5 years. These specimens were kept in small glass tubes in a laboratory cupboard at room temperature but were not made moisture-proof by being placed in a desiccator. Experiments made on *Lupinus albus* in 1933 show much less toxicity than those made with the same material in 1928.

Inasmuch as most snake venoms effect hemolytic changes, it was interesting to inquire into the phytotoxic properties of a number of hemolytic drugs obtained from the vegetable kingdom. Experiments were therefore made with solutions of solanin, saponin and

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* The cobra venom used in these experiments was obtained from Professor R. N. Chopra, School of Tropical Medicine, Calcutta; the other venoms were acquired through the courtesy of Dr. T. S. Githens, of the Mulford Biological Laboratories.

TABLE I.

	1928 Experiments					1933 Experiments
	1:50,000 (Fresh emulsion at 19°C.)	1:25,000 (Fresh emulsion at 19°C.)	1:10,000 (Fresh emulsion at 19°C.)	1:25,000 (After standing 24 hours at 24°C.)	1:10,000 (After standing 48 hours at 24°C.)	1:10,000 (Fresh emulsion at 19°C.)
	%	%	%	%	%	%
Crotalus atrox	86	64	44	88	71	70
Crotalus exsul	86	60	30	83	60	36
Bothrops atrox	82	64	18	88	63	55
Ankistrodon piscivorus	90	70	12	83	78	56
Naja (cobra)	—	53	32	—	—	—
Solanin	128	117	110	—	—	—
Saponin	127	120	120	—	110	—
Digitonin	120	100	98	—	93	—

digitonin. All the vegetable toxins hemolyzed blood corpuscles to a greater extent than did the snake venoms. When solutions of these vegetable drugs were tested on the growth of *Lupinus* seedlings, however, it was found that they caused practically no inhibition in growth. These findings agree with those from previous studies by the writer, which indicated that poisons obtained from the animal world are relatively non-toxic for plant protoplasm; and, vice versa, that poisons coming from the vegetable kingdom are usually more poisonous for animals than for plants.[6]

6765

Effect of Ultraviolet Rays and of Methylthionine Chloride and Heparin on Snake Venoms.

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It is well known that snake venoms in solution or suspension rapidly decompose on standing at room temperature, but few investigators have studied the effect of light on such solutions. Faust, in

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[6] Macht, *Science*, 1930, **71**, 302.

Heffter's *Handbuch der Pharmakologie*, cites only 2 papers, that of Noguchi,[1] who studied the phytodynamic action of eosin and erythrosin on snake venoms in the dark and in sunlight, and of Massol,[2] who found that exposure to rays of a quartz mercury vapor lamp diminished the potency of cobra venom to a greater extent than did its antitoxin. The writer wishes to report experiments on the toxicity of snake venoms exposed to mercury vapor lamps. Solutions or suspensions of dry venom were made in saline and exposed in small, transparent quartz containers to the rays of a Kromayer lamp. The toxicity of the irradiated and control, or non-irradiated, solutions of the venoms* was studied by zoopharmacological methods on mice and on plants by phytopharmacological methods in the manner described in the preceding article.

TABLE I.
Effect of Venoms on Growth of *Lupinus albus*.

Venom of	Concentration	Phytotoxic Index of Fresh Emuls.	Phytotoxic Index of Emulsion exposed to Kromayer Lamp for 15 min.
		%	%
Crotalus exsul	1:10,000	36	77
Bothrops atrox	1:10,000	55	100
Ankistrodon piscivorus	1:10,000	54	100
Naja (cobra)	1:10,000	32	88

Table I shows the effect of exposure of venom solutions to a Kromayer lamp for 15 minutes. The poisons of the *Crotalus exsul*, *Bothrops atrox*, *Ankistrodon piscivorus* and *Naja* (cobra) were studied in this manner. It will be noted that their toxicity for *Lupinus albus* seedlings was enormously diminished by exposure to ultraviolet rays.

Experiments with irradiated and control solutions of the same venoms were made on mice by intraperitoneal and subcutaneous injections; and it was found that in the zoopharmacological experiments also the potency of the irradiated specimens was greatly diminished.

These findings with exposure of venom emulsions in quartz containers to ultraviolet rays suggested an inquiry regarding the effect of irradiation of animals with fresh venom. Such experiments were carried out on mice. It was found that mice injected subcutaneously, and even intraperitoneally, with rattlesnake venom lived much longer when irradiated with ultraviolet light than the non-irradiated controls injected with the same quantity of venom. Table II gives the results obtained in 6 experiments with the venom of *Crotalus atrox*. However, similar experiments with cobra venom on mice exposed to ultraviolet rays revealed little diminution in its potency, indicating, as Noguchi has already noted, that different kinds of venom vary markedly in their chemical composition.

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[1] Noguchi, *J. Exp. Med.*, 1906, **8**, 252.

[2] Massol, *Compt. rend de la Soc. de Biol.*, 1911, **71**, 183.

* The cobra venom used in this investigation was obtained from Professor R. N. Chopra, School of Tropical Medicine, Calcutta; and the other venoms were acquired through the courtesy of Dr. T. S. Githens of the Mulford Biological Laboratories.

TABLE II.

Venom of *Crotalus atrox* injected in mice.

1. Mouse, wt. 24 gm., injected subcutaneously with 0.5 mg., receiving no irradiation, died in 1 hour and 45 min.
2. Mouse, wt. 20 gm., injected subcutaneously with 0.5 mg., irradiated with Alpine Sun Lamp for 30 min., died in 5 hours and 44 min.
3. Mouse, wt. 24 gm., injected subcutaneously with 0.5 mg., receiving no irradiation, died in 3 hours and 7 min.
4. Mouse, wt. 20 gm., injected subcutaneously with 0.5 mg., irradiated with Alpine Sun Lamp for 30 min., died in 22 hours.
5. Mouse, wt. 20 gm., injected intraperitoneally with 1.0 mg., receiving no irradiation, died in 22 min.
6. Mouse, wt. 24 gm., injected intraperitoneally with 1.0 mg., irradiated with Alpine Sun Lamp for 15 min., died in 31 min.

Brooks[3] and Hanzlik[4] have recently called attention to the beneficial effects of methylene blue injections in case of carbon monoxide and cyanide poisoning, and Geiger[5] actually reported favorable clinical results obtained by such treatment. This discovery prompted the writer to study the effect of methylene blue on snake venoms. Tests were made with the venoms of *Crotalus exsul, Bothrops atrox, Ankistrodon piscivorus* and *Naja* both by the phytopharmacological method on *Lupinus albus* seedlings and by the zoopharmacological method on various animals. It was found that a combination of the venom with methylene blue, 1:50,000, did not diminish the toxicity of the poison for *Lupinus* seedlings. Similarly, injections of venoms with and without methylene blue into mice, rats, guinea pigs and pigeons, by subcutaneous, intramuscular, intraperitoneal and intravenous routes, revealed that no antagonistic or neutralizing effect was produced by the dye. The experiments indicated, if anything, a slight increase in toxicity of the venoms.

Inasmuch as snake venoms are complex mixtures of various principles, some of them being in the nature of hemagglutinins and thrombokinase, it was deemed desirable to perform some experiments regarding the effect of heparin on snake poisons. A very potent preparation of heparin made in these laboratories was employed; and the toxicity of snake venoms with and without heparin was tested both on seedlings of *Lupinus albus* and on mice. The experiments were made with venoms of *Crotalus atrox* and of *Naja*. Neither the phytopharmacological nor zoopharmacological experiments revealed any difference in the toxicity of the venoms produced by their combination with heparin.

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[3] Brooks, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1228.

[4] Hanzlik, *J. Am. Med. Assn.*, 1933, **100**, 357.

[5] Geiger, *J. Am. Med. Assn.*, 1932, **99**, 1944.

Summary. 1. Ultraviolet radiations from a mercury vapor quartz lamp produce rapid deterioration of the potency of snake venoms, as indicated by both zoopharmacological and phytopharmacological tests. 2. Methylthionine chloride, or methylene blue, does not affect the toxicity of snake venoms for either living animals or plants. 3. Heparin injections produce no change in the toxicity of snake venom, as tested on living animals or plants.

6766

Specific Inhibition of Bacteriophage by Bacterial Extracts.

PHILIP LEVINE AND ARTHUR W. FRISCH. (Introduced by Paul F. Clark.)
With the technical assistance of Marion L. Rohn.

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There is as yet no final explanation for the specificity involved in bacteriophage action. Burnet[1] claims on the basis of experiments made with the Salmonella group of organisms that the action, in a general way, parallels the serologic definition of the heat stable factors. However, he recognized that several observations made by himself and others are not compatible with this point of view. One of these is the failure to demonstrate inhibition of phage action by means of extracts of bacteria containing specific carbohydrate material.*

In our experiments positive results were obtained using saline extracts prepared according to the method of Furth and Landsteiner,[3] except that no alkali was employed. The 2 phages employed, anti-dysenteriae Shiga and anti-B. paratyphosus B were derived from chicken stool filtrates. They were specific in their reactions when tested against these 2 organisms; the anti-B. paratyphosus B phage, however, acted also on *B. aertrycke.*

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[1] Burnet, F. M., *J. Path. and Bact.*, 1930, **33**, 647.

* See also Prausnitz and Firle.[2]

[2] Prausnitz, C., and Firle, E., *Centr. f. Bact.*, 1924, **93**, 148.

[3] Furth, J., and Landsteiner, K., *J. Exp. Med.*, 1929, **49**, 727.

The tests were made by mixing serial dilutions of phage in a volume of 0.5 cc. with 0.5 cc. of a 1% solution of the extracts previously run through a Berkefeld filter and incubating the mixture overnight at 37° and 24 hours in the ice-chest. To each of the tubes were added 4 cc. of broth and a few drops of a young suspension of the homologous or test organism. Readings were made on the basis of the amount of growth. The signs ftr, tr, ±, +, +±, indicate increasing degrees of turbidity; the letters cl. indicate complete clearing.

TABLE I.

Tests with B. dysenteriae Shiga.

Dilutions of Anti-Shiga Phage	10^{-3}	10^{-5}	10^{-7}	10^{-9}	10^{-11}	10^{-13}	10^{-15}
Incubated with Shiga extract	+	+	+	+	+±	+±	+±
	tr	+	+±	+±	+±	+±	+±
	cl	cl	+±	+±	+±	+±	+±
	cl	cl	cl	+±	+±	+±	+±
Aertrycke extract	ftr	tr	±	+	+±	+±	+±
	cl	cl	tr	ftr	+	+±	+±
	cl	cl	cl	cl	+	+±	+±
	cl	cl	tr	cl	tr	tr	+
Saline	cl	tr	±	+	+	+	+
	cl	cl	cl	ftr	+	+±	+±
	cl	cl	cl	cl	cl	+±	+±
	cl	cl	cl	cl	cl	cl	+±

1st reading made after 5 hours at 37°.

2nd reading made after 2 additional hours at 37° and 5 hours at room temperature.

3rd reading made after standing overnight at room temperature.

4th reading made after an additional 24 hours at room temperature.

It is seen from Table I that the action of the phage against *B. dysenteriae* Shiga was inhibited by saline extracts of the homologous organism but only to a slight degree by a similar product derived from *B. aertrycke.* Thus in the tubes containing the greater concentration of Shiga phage its action in the homologous mixture is delayed while in the latter tubes of the series the fixation of the phage was complete. The specific inhibition in the case of the Shiga phage was evident even after the tests stood for 4 days at room temperature.

To demonstrate further the specificity of the reaction, tests were made with the phage against *B. paratyphosus B* and extracts of *B. aertrycke* which shares with the former organism the same heat stable factors. This combination was employed because a sufficiently potent anti-aertrycke phage was not available, and because the anti-*B. paratyphosus* B phage, as previously mentioned, was lytic for *B. aertrycke*. In these tests made with *B. paratyphosus* B (Table II) the action of the phage was inhibited by an extract of *B. aertrycke* but hardly at all by that of *B. dysenteriae* Shiga.

TABLE II.

Test with B. paratyphosus B.

Dilution of Anti-*B. paratyphosus* Phage	10^{-5}	10^{-7}	10^{-9}	10^{-11}	10^{-13}	10^{-15}	10^{-17}	10^{-19}
Incubated with Shiga extract	cl	tr	+	+±	+±	+±	+±	+±
	cl	cl	cl	tr	±	+	+±	+±
	cl	cl	cl	cl	cl	±	+±	+±
	cl	cl	cl	cl	ftr	tr	+±	+±
Aertrycke extract	±	+	+±	+±	+±	+±	+±	+±
	±	+	+±	+±	+±	+±	+±	+±
	±	±	±	+	+±	+±	+±	+
	+	±	±	±	+	+±	+±	tr†
Saline	cl	cl	±	+	+±	+±	+±	+±
	cl	cl	cl	cl	tr	+±	+	+±
	cl	cl	cl	tr	tr	+±	tr	+±
	cl	tr	cl	cl	cl	tr	cl	+±

1st reading made after 4 hours at 37°
2nd reading made after 3 additional hours at room temperature.
3rd reading made after 1 additional hour at room temperature.
4th reading made after 4 additional hours at room temperature.

† This discrepancy may perhaps be due to some irregularity in the titration. In these experiments, however, care was taken to avoid inaccurate transfer of active agent in the titrations, by washing out the pipettes or changing them after a number of dilutions.

While the phage active against *B. paratyphosus* B had approximately the same titer as the anti-Shiga phage, rapid overgrowth occurred so that the specific effect was seen only during the 12 hours after the addition of the bacteria. Although in the readings made 24 hours later, the tubes showed equal degrees of turbidity, the specific inhibition could still be verified in some of the mixtures by the observation that in those tubes containing the heterologous extract or saline (for instance, Table II, dilution 10^{-13}) the turbidity was apparently due to organisms resistant to the phage; in the tube containing homologous extract, however, the bacteria, never having been lysed, remained sensitive to fresh addition of the lytic agent.

The inhibition of the *B. paratyphosus* phage by extracts of *B. aertrycke* was evident also when *B. aertrycke* was used as a test organism.

The effect described was observed in numerous tests but the degree of inhibition was somewhat variable. Experiments are under way to determine the optimal conditions required for the reaction. In any event it is essential to observe the course of the reaction over a number of hours. This is so especially in the case of the tests with the anti-*B. paratyphosus* B phage.

It remains to be established why Prausnitz and Burnet failed in their experiment to obtain inhibition of the phage by means of bacterial extracts. Two factors that may possibly serve as an explanation are their use of anti-formin extracts or too short a period of contact of phage and extract.

Particular significance is attached to the inhibition of the anti-*B. paratyphosus* B phage by extracts of *B. aertrycke* since the 2 organisms have the same heat stable components. Experiments along this line seem to offer a general method for the investigation of the specific behavior of the bacteriophage.

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Clinical Manifestations of Variations in Blood Magnesium—Hypomagnesaemia and Hypermagnesaemia.*

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Serles and one of us reported[1, 2] that, contrary to the usual conceptions, a large part of the magnesium ingested orally as magnesium sulphate is absorbed from the intestine and in normal animals and normal human beings about 40% of the ingested magnesium passes out in the urine in 24 hours, without causing any significant rise in plasma magnesium. The determinations of magnesium were facilitated by the introduction of a modification of Kolthoff's colorimetric method[3] for determining the magnesium in the oxalated Ca-free

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* This investigation was supported by a grant from the Therapeutic Research Fund of the American Medical Association.

[1] Hirschfelder, A. D., and Serles, E. R., *J. Clin. Invest.*, 1932, **11**, 841.

[2] Hirschfelder, A. D., and Serles, E. R., *J. Pharmacol. and Exp. Therap.*, 1932, **45**, 264.

[3] Kolthoff, I. M., *Biochem. Z.*, 1927, **185**, 344.

plasma and in oxalated Ca-free urine, after precipitation of the phosphate by uranyl acetate. We then found that in nephrectomized or nephrotic animals, oral administration of magnesium sulphate caused the plasma magnesium to rise significantly, a single dose bringing it to about 10 mg. per 100 cc. plasma, larger doses to above the coma producing level (which is about 17 mg. per 100 cc.).

Extending these studies to human beings we have obtained similar results.

1. High blood magnesium. This was found especially in nephritics after the administration of Epsom salts. In normal individuals 20-30 gm. $MgSO_4$, 7 H_2O by mouth does not significantly raise the blood magnesium and about 40% is excreted in the urine in 24 hours. In nephritics less of the ingested Mg is excreted in the urine, and the blood magnesium rises.

20-30 gm. $MgSO_4$, 7 H_2O administered to 9 patients with acute and chronic glomerulo nephritis raised blood magnesium from near normal to 9.8-11.3 mg. All these patients were definitely more drowsy when at these levels. Two nephritics with blood magnesium 8.9-9.0 without administration of $MgSO_4$ were definitely drowsy and unresponsive.

Coma is induced in animals when the blood magnesium approaches 17 mg. Mg per 100 cc. Elevation of blood magnesium to about two-thirds of the coma-producing level thus seems to be accompanied by a tendency to somnolence.

It is probable that repeated purgative doses of Epsom salts by mouth can induce coma in nephritis, and that many cases of supposed uremic coma are really magnesium coma induced by Epsom salts. From this coma animals and probably patients can be awakened and their lives prolonged by intravenous calcium chloride.

2. Low blood magnesium (0.9 to 1.37 mg.) was found in 10 cases, all manifesting twitching or convulsions. One of these was parathyroid tetanic, two epilepsy, one cerebral injury, the others acute or chronic nephritis. Three of the latter were given Epsom salts by mouth, blood magnesium rose to 3.3 to 5.8 mg. Mg, twitchings or convulsions disappeared.

There thus seems to be a definite clinical syndrome of twitching or convulsions associated with low blood magnesium.

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A Method for Determining the "Permeability Ratio" for Water and a Solute.

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In an earlier paper[1] it was shown that the simultaneous determination of permeability constants for water and for a dissolved substance is possible by a study of the volume changes of a cell placed in an originally isotonic medium to which the penetrating substance has been added in known amount. The method depends on measuring the shrinkage of the cell and the time of attainment of the minimum volume, the values of the permeability constants in question being read off from a prepared chart. The published chart was, however, restricted in its usefulness to a single concentration of the penetrating substance.

In cases, which are frequently encountered, where a knowledge merely of the ratio of the 2 permeability constants is desired, a more direct and general solution of the problem is possible, since the relation between the ratio, K, of the 2 constants, the external concentration, C_s, of the penetrating solute, the amount, S, of the solute that has penetrated the cell, and the volume, V, of the latter may be shown to be:

$$\ln [K(S - C_s)^2 - (KC_s + K - 1)(S - C_s)(V - 1) - C_s(V - 1)^2]$$
$$- \frac{KC_s + K + 1}{\sqrt{A}} \ln \frac{2K(S - C_s) - [(KC_s + K - 1) + \sqrt{A}](V - 1)}{2K(S - C_s) - [(KC_s + K - 1) - \sqrt{A}](V - 1)} = \ln KC_s^2$$

where $A = K^2(C_s + 1)^2 + 2KC_s - 2K + 1$.

This equation is obtained by solving, subject to the condition that when $V = 1$, $S = 0$, the differential equation

$$\frac{dV}{dS} = \frac{KS + K - (C_s + 1)V}{V - S}$$

which is in turn derived from equations 2 and 3 of the earlier paper, using such units of concentration and volume that a and C_m in these equations are both equal to unity.

As shown previously,[2] the condition for minimum volume in the case in question is that $S = (C_s + 1)V - 1$. Substituting this value of S in the general equation, simplifying, and changing for convenience to common logarithms, we obtain finally

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[1] Jacobs, M. H., *J. Cell. and Comp. Physiol.*, 1933, **2**, 427.

[2] Jacobs, M. H., and Stewart, Dorothy R., *J. Cell. and Comp. Physiol.*, 1932, **1**, 71.

$$\log (V_{min} - 1)^2 = \log KC_s^2 + \frac{KC_s + K + 1}{\sqrt{A}} \log \frac{KC_s + K + 1 - \sqrt{A}}{KC_s + K + 1 + \sqrt{A}}$$

From this relation the minimum volume corresponding to any values of K and C_s may readily be calculated, remembering that for the case in question only the value of V_{min} which is less than unity has a real physical significance, since it is impossible for the cell volume ever to exceed its initial value. Conversely, for any given value of C_s the value of K may be calculated from the observed minimum volume. Practical applications of this principle to problems of cell permeability will be discussed in a later paper.